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CHEMICAL ASPECTS OF NITROGEN FIXATION IN SOILS AND CROP PRODUCTION*

By N. R. Dhar

I am very grateful to the Council of the Indian Chemical Society, and specially Prof. P. Rây, for inviting me to deliver the first lecture in commemoration of our great Guru, Acharya Prafulla Chandra Ray. I had the good fortune to be closely associated with him since 1907 till his death in 1944. I was initiated by him in 1911 in investigations on the physical properties of nitrites and hyponitrites, and we published a series of joint papers on this line in the Journal of the English Chemical Society. These investigations have now formed a part of the literature in Physical and Inorganic Chemistry. My Guru had the impression that electric conductivity and other physical properties could not be determined in India at that time and it would be necessary for him to go to England to obtain the "Conductivity" water needed for these experiments. A little later, with the help of my friends, Shri D. N. Bhattacharya, Shri A. K. Dutta and others, we determined the transport number of the nitrite ion by the electrolysis of silver nitrite solutions at different concentrations, dissociation constant of nitrous acid and the kinetics of the reaction between iodine and nitrite, both in light and in the dark, and the action of nitric acid on mercury, copper, zinc etc. and several reducing agents. Hence, I followed the footsteps of my Guru in the study of nitrite chemistry.

For over 25 years we have investigated the problem of nitrogen fixation and nitrogen loss with a band of able young men in the Chemical Laboratories of the University of Allahabad and the Sheila Dhar Institute of Soil Science with the ultimate view to ease the world food situation by increasing land fertility as cheaply as possible. Our Guru used to say that " *Anna Chinta Chamatkara*" "अन्नचिन्ता चमत्कारा", supply of food is the real problem of our country.

In this connection it will be of great interest to record here the remarks of the late Prof. H. E. Armstrong in his article on 'Future of Chemistry in India' in the Acharya Prafulla Chandra Ray Memorial Volume (1932, pp. 15-16).

"The great work of the future, however, specially in India, will be the development of agriculture with the definite object in view of providing food of approved value, far higher in quality than that now produced. Only in recent years have we been able to set this before us as our object."

"We have failed thus far, even in England, to place Agriculture upon a pedestal of the highest scientific endeavour. We have had no efficient College to this end. I can imagine no higher service to India than the establishment of such an institution. Only a potential Liebig will be able to bring it into being and supervise its operations.

*First Acharya Prafulla Chandra Ray Memorial Lecture, delivered on August 2, 1948 in Calcutta.

Twenty years hence, perhaps, such a leader may be forthcoming, if meanwhile a few men who feel that they have some call to such service, some biological feeling, will set themselves in training, disregarding academic traditions and forswearing all desire to advertise that they have knocked another spot off another atom or in some other way, of remote concern to the world, made themselves exceptional."

"Competent chemists, they will carry on their studies, both in field and laboratory, in every possible and desirable direction, so as to secure a commanding knowledge of the problems of animal and plant life and of the soil. Only men so qualified, with ripened powers of imagination, will be competent to act as saviours of the people in the not distant future. The example Sir Prafulla Chandra Ray has set may well serve to encourage such an order into being. They will be the scientific missionaries of the future, sworn to social service alone."

Although organic matter has been used as a manure from times immemorial, and even today 97% of world food production has to be attributed to the humus of the soil—3% being due to the application of artificial nitrogen—the functions of organic matter in the soil have not been properly cleared up.

Fixation of Atmospheric Nitrogen in Soil by adding Organic Matter

About 25 years ago over 100 sugar factories were newly started in India and 100,000 tons of molasses, which is a by-product of the industry, were practically wasted. We started a large number of experiments on the use of molasses as a manure for normal and alkaline soils. From our experiments, which are reproducible under European conditions with a different time factor, we concluded that there was considerable nitrogen increase when molasses was mixed with soil or sand and allowed to undergo slow oxidation in air, both in sterile and unsterile conditions. In every case the initial, total and available nitrogen of the mixture were determined and it was observed that after 3 or 4 weeks, the ammoniacal and total nitrogen increased markedly and the carbon of the system decreased. We also observed that increase of nitrogen (total and ammoniacal) was more pronounced when the system received sunlight or artificial light than when kept in dark. From these experiments we came to the conclusion that molasses, when added to soil or sand, fixed atmospheric nitrogen more in light than in dark. Nitrogen fixation is much greater in sand in which the initial nitrogen is lower than in soil.

As molasses cannot be obtained in other countries for manuring fields, we carried on experiments with other energy-producing materials like sugars, fats, oils, straw, leaves, paper, cowdung, lignin, sawdust, lignite and coals. In the case of molasses, sugars, glycerol and other readily-soluble energy materials, oxidation of carbonaceous substances and nitrogen fixation are more rapid than with other types of energy materials, and the total and ammoniacal nitrogen go on increasing from the very beginning ; but with the other group of energy-producing materials, the increase of total nitrogen (and not ammoniacal nitrogen) takes place up to 4 or 6 months. After this period there is an increase of ammoniacal nitrogen also in the system. A few of our experimental results with straw, cowdung and coal are recorded below.

TABLE I

Experiments with straw.

100 g. of soil + 3.75 g. of wheat straw.

Date.	Total C in 100 g soil.	Total N in 100 g. soil.	*Efficiency.
	Unsterile (exposed to sunlight).		
28—6—47	1.886 g.	0.0622 g.	...
6—1—48	1.571	0.0698	24.8
18—3—48	1.442	0.0730	24.6
28—6—48	1.281	0.0765	24.6
	Covered (dark).		
28—6—47	1.886	0.0622	...
6—1—48	1.735	0.0639	11.2
18—3—48	1.663	0.0647	11.1
28—6—48	1.569	0.0657	10.6
	100 g. of soil + 2.5 g. of wheat straw.		
	Unsterile (exposed to sunlight).		
28—6—47	1.386	0.0544	...
6—1—48	1.091	0.0619	25.4
18—3—48	0.975	0.0648	25.0
28—6—48	0.851	0.0678	24.0
	Covered.		
28—6—47	1.386	0.0544	...
6—1—48	1.243	0.0662	12.4
18—3—48	1.181	0.0569	11.3
28—6—48	1.100	0.0578	11.0

*Nitrogen fixed in mg. /g. of carbon oxidised.

TABLE II

Experiments with cowdung.

250 g. of soil +40 g. of cowdung.

Date.	Total C in 100 g. soil.	Total N in 100 g. soil	†Azotobacter count.	*Efficiency.
		Unsterile (light).		
3—1—47	1.476 g.	0.0931 g.	2.5	...
5—3—47	1.212	0.0986	18.0	21.0
3—5—47	1.001	0.1029	39.0	20.0
		Unsterile (dark).		
5—1—47	1.460	0.0924	2.5	...
7—3—47	1.310	0.0942	72.0	11.8
5—5—47	1.181	0.0955	135.0	10.0
		Sterile (light).		
8—1—47	1.442	0.0916	...	...
10—3—47	1.277	0.0941	...	15.4
9—5—47	1.139	0.0962	...	15.0
		Sterile (dark).		
10—1—47	1.460	0.0924	...	...
11—5—47	1.285	0.0939	...	8.2

†In million per g. of dry soil.
*N fixed in mg./g. of carbon oxidised.

TABLE III

Experiments with coal.

The mixture was expospd to sunlight in dishes from April 10 to August 10, 1950 and then analysed. It was daily stirred and kept moist with distilled water.

		State.	C initially present in 100 g. of sand.	C oxidised in 100 g. of sand after exposure for 250 hrs. (April 10 to Aug. 10).	Total N initially present in 100 g. of sand.	Gain in total N per 100 g. of sand after exposure.	Efficiency.
				Unsterile.			
Sand+ Indian lignite	250 g. 2%	Exposed.	1.2732 g.	0.1922 g.	15.26 mg.	4.28 mg.	22.26
		Covered.	1.2732	0.1300	15.26	1.29	9.91
Sand+ Indian lignite	250 g. 1%	Exposed.	0.6366	0.0951	7.63	1.999	21.02
		Covered.	0.6366	0.0616	7.63	1.827	13.42
Sand+ Indian lignite +wheat straw	250 g. 2% 2%	Exposed.	2.0461	0.4819	28.46	9.51	19.03
		Covered.	2.0461	0.4509	28.46	4.49	9 95
Sand+ Indian lignite +wheat straw	250 g. 1% 2%	Exposed.	1.4096	0.3996	20.85	7.96	19.91
		Covered.	1.4096	0.3766	20.85	4.05	10.75
Sand+ Assam coal	250 g. 2%	Exposed.	1.5756	0.0662	26.40	1.03	15.55
		Covered.	1.5756	0.0226	26.40	0.19	8.40
Sand+ Assam coal	250 g. 1%	Exposed.	0.7878	0.0321	13.20	0.49	15.26
		Covered.	0.7878	0.0178	13.20	0.13	7.30
Sand+ Assam coal +wheat straw	250 g. 2% 2%	Exposed.	2.3485	0.0185	39.62	7.32	17.49
		Covered.	2.3485	0.3860	39.62	4.19	10.85
Sand+ Assam coal +wheat straw	250 g. 1% 2%	Exposed.	1.5608	0.3753	26.42	7.67	20.44
		Covered.	1.5608	0.3558	26.42	4.21	11.83

Moreover, by adding repeated doses of municipal rubbish with a C/N ratio much greater than 10 to our soils containing 0.04% total nitrogen, the nitrogen status has been raised to 0.25% and bumper crops have been obtained in these improved lands.

In the Presidential Address to the National Academy of Sciences, India, on 19th December 1935, I concluded from a large amount of experimental observations that the chief function of organic matter added to the soil was fixation of nitrogen. In numerous subsequent publications I have developed the same theme from experiments with all types of energy materials including coals. I have also emphasised that a mixture of organic substance with artificial nitrogenous matter is a better manure than ammonium salts alone. Some of our results are recorded below.

TABLE IV

Amount of nitrogen fixed in light per g. of carbon oxidised.

	Sterile.				Unsterile.			
	Original N in the system in per cent.				Original N in the system in per cent.			
	0.	0.001.	0.05.	0.08.	0.	0.001.	0.05.	0.08.
Glucose	38	28	13	9	40	29	14	10
Cane sugar	38	27	12	8	39	28	14	10
Mannitol	37	28	12	8	40	28	13	9
Starch	40	28	13	9	40	29	14	10
Glycerol	39	28	12	9	38	28	13	9
Butter	38	28	11	8	39	29	13	9
Cellulose	40	30	14	10	41	30	13	10
Lignin	34	26	11	7	33	25	12	...
Coal	25	20	...	...	26	21	...	...

In the absence of light the fixation of nitrogen is less than half of that in light. In the system with no initial nitrogen, pure oxides of iron, zinc, manganese, nickel, aluminium etc. were used instead of soil. In these experiments either there was no phosphate present in the system or the percentage of P_2O_5 did not exceed 0.075, *i. e.* the phosphate status was low.

On obtaining these results we consulted the literature on the subject and found that on adding farmyard manure to Rothamsted soil, marked increase of nitrogen was reported, and hence, in my Address, referred to above, I stated as follows:

"Russell ("Soil Conditions & Plant Growth", 1932, pp. 76-78, 313, 361) has reported that the yield of barley and straw with farmyard manure in Rothamsted field experiments from 1852-1922 is better than that obtained with complete artificial manures containing ammonium salts, potassium salts and phosphates. This is due to the fact that there is more nitrogen in the field manured with cowdung than with artificials, as is evident from the following data from the Rothamsted fields:

	Total N%.
(1) Receiving no manure since 1843	0.095
(2) Receiving farmyard manure since 1852	0.256
(3) Receiving complete artificials & $(NH_4)_2SO_4$	0.09
(4) Receiving potash & phosphate but no nitrogen	0.090 ....."

After a few years I learnt that marked increase of soil nitrogen had also been reported in the Missouri Experiment Station by the use of barnyard manure. Similarly in Lyngby (Denmark) the following results were obtained.

TABLE V

% Nitrogen in air-dry soil.

	Unmanured.	Farmyard manure.	% Nitrogen increase.
Askov loam-field 1942	0.106	0.130	22.6
Askov sand-field 1942	0.066	0.086	30
Lyngby 1933	0 146	0.169	15.7

Moreover, E. M. Crowther, in his paper on "The Study of Soil Organic Matter & Organic Manures by means of Field Experiments" has recorded the following results:

TABLE VI

Nitrogen contents of Brodbalk soils, Rothamsted (metric tons per ha.). Gain over unmanured plot in relation to amount of nitrogen previously added as farmyard manure.

"Original N in 1843 : 0.122%".

	Plot 2B.				Plot 2A.		
	Gain.	Added.	Gain as % of added.		Gain.	Added.	Gain as % of added.
1865	1.8	4.9	37	...	...	...	...
1881	2.1	8.5	25	...	...	...	...
1893	3.0	11.2	27	1893	1.0	2.0	50
1914	4.0	15.9	25	1914	2.5	6.7	37
1936	3.1	20.2	15	1936	2.1	10.7	20
1945	3.3	22.4	15	1945	2.2	12.9	17

From Crowther's paper it will be seen that the gain in total nitrogen in the Rothamsted plots has been more marked in the beginning of the experiments than in later periods. This cannot be attributed, as is generally done, only to the retention of the nitrogen added in the form of farmyard manure. The original soil nitrogen in Rothamsted was 0.122% in 1843 when manuring experiments were first started. In the first 22 years there was more marked increase of soil nitrogen (46%) than in the next 28 years (21%). If the retention of the added nitrogen were the cause of the increase of total nitrogen observed, this behaviour should have been more marked in the later period of the experiments when the total nitrogen content of the soil became high. It is well known that when organic matter is added to the soil of low humus content, the added organic matter is oxidised very rapidly in the beginning. Hence, our conclusion that cowdung or farmyard manure not only supplies the nitrogen it contains but it also fixes atmospheric nitrogen by utilising the energy obtained from the oxidation of carbohydrates, pentosans and other compounds present in farmyard manure, can explain the Rothamsted, Missouri and Lyngby results where more marked nitrogen increase has been reported in soils containing smaller amounts of total nitrogen.

HUMUS NITROGEN DUE CHIEFLY TO NITROGEN FIXATION

It is estimated that 35 billion kilograms, *i. e.* 34750 million tons of cellulose containing 13750 million tons of carbon are added every year to the earth. From our experiments we find that 40% of the carbon is oxidised in our climatic conditions in 4 or 5 months, and even if the same amount be taken to be oxidised on the surface of the whole earth in one year, it would mean that 5500 million tons of carbon are oxidised every year. On a moderate estimate of 15 mg. nitrogen fixation per gram of carbon oxidised in sunlight, about 82.5 million metric tons of nitrogen are added to the earth by fixation provided that 40% of the carbon added is oxidised. We can conclude that out of the total 82.5 million tons, at least 50%, *i.e.* 41.25 million tons of nitrogen are fixed in soils by absorption of solar light. The total output of nitrogen fixed synthetically in the factories of the world in 1937 was 3.54 million tons, *i.e.* 1/12 th of what is obtained by fixation under natural conditions by the utilisation of sunlight.

It is interesting to note that although the velocity of oxidation of organic matter in sterile conditions is smaller than in unsterile conditions, the efficiency of nitrogen fixation in sterile conditions is of the same order as that obtained in the unsterile experiments, as is clear from the following data.

TABLE VII

Condition.	Material used.	Period of exposure. (months).	Carbon oxidised during the whole month.	Carbon oxidised during one month.	Amount of N fixed per 100 g. of soil.	Efficiency in sunlight.
Unsterile	Cowdung.	4	0.475%	0.119%	9.8 mg.	20.7
Sterile	Do	4	0.303	0.076	4.6	15.2
Unsterile	Neem leaf	4	0.402	0.101	9.1	22.6
Sterile	Do	4	0.221	0.055	2.8	17.2
Unsterile	Wheat straw	12	0.605	0.050	14.2	23.8
Sterile	Do	12	0.319	0.026	5.5	17.4

FORMATION OF FERTILE SOIL FROM EARTH'S CRUST

We have obtained marked nitrogen fixation when energy materials are mixed with oxides of iron, nickel, aluminium, etc. containing no initial nitrogen, more in light than in the dark. The higher value of efficiency of nitrogen fixation obtained with oxide surfaces as compared with that obtained in soils is due to the fact that the phenomena of nitrogen fixation and nitrogen loss go on simultaneously, the fixation process is being opposed by the loss due to nitrification. The unstable substance, ammonium nitrite, is formed in the process of oxidation involved in the nitrification of proteins and other nitrogenous compounds produced by fixation or originally present in the system. But in soils there is already a certain amount of combined nitrogen, *i. e.* to the extent of 0.04 to 0.05% in tropical soils, and hence, the loss is more marked when oxide surfaces are used containing no nitrogen than in the soils containing nitrogen. These observations are of fundamental importance in explaining the evolution of fertile soils from the parent rocks of geological ages. The geological deposits, *i. e.* parent materials of soils, do not contain any organic matter but can contain inorganic nitrogen in the form of nitrates or of ammonium salt in very small quantities. The nitrates can, under the influence of light and moisture and seeds, form the first set of vegetable or plant life or alga, the nitrogen need of which is met from the inorganic nitrogen originally present in rocks in smaller quantities. The carbohydrates or cellulosic materials or other energy materials formed in photosynthesis undergo decomposition in course of time and are oxidised causing nitrogen fixation in this process which is aided markedly by light absorption, and thus the store of the nitrogen in the system is increased. This in its turn leads to a more abundant growth of vegetation, and this process goes on in which the carbon and nitrogen status of the system is improved leading to the formation of a fertile soil. This nitrogen fixation, which in the beginning at any rate is a non-biological surface reaction, is aided by light absorption owing to utilisation of light in this process. As the original mineral is poor in nitrogenous compounds, the nitrogen fixation is greatly enhanced in the beginning, but with the storing up of nitrogen the efficiency falls off, and thus the nitrogen and

carbon status of the soil reaches a maximum limit depending on the climate of the region. As a matter of fact, much more nitrogen is fixed in soils in this manner by light absorption than the amounts fixed in the industrial processes taken together.

The organic matter in the soil may also be caused by the growth of alga. It is believed to be valuable in most circumstances, but on newly formed soil it is of the greatest importance. The carbon of the algae when decaying is oxidised and the energy liberated leads to the fixation of the atmospheric nitrogen.

The residual effect of farmyard manure, as observed throughout the whole world, may be due not only to the conservation of nitrogen, as hitherto believed, but is caused by the fixation of atmospheric nitrogen through oxidation of the energy materials like pentosans, celluloses, fats, etc., and aided by the absorption of sunlight. It seems that wherever the residual effect of a manure has been observed, e.g. with molasses, hay or cowdung or farmyard manure, it is chiefly due to nitrogen fixation in the soil, and perhaps no residual effect will be observed with a manure which is incapable of fixing atmospheric nitrogen on the soil surface although it may contain carbon. It seems that so far no worker has reported that legumes leave as much residual effect as cowdung or farmyard manure or molasses.

Tropical Soil contains more available but less total Nitrogen than Temperate country Soils

In Indian soils under cultivation as well as in those of other tropical countries, the total nitrogen is much lower than that in the soils of temperate countries. This is due to the fact that the prevailing high temperature and intensity of sunlight accelerate oxidation of carbonaceous and nitrogenous compounds in tropical soils, thus entailing a loss of nitrogen caused by nitrification of protein and other nitrogenous compounds. But when the land is covered with grass or other vegetation, this oxidation can be retarded and loss of nitrogen diminished with the result that the nitrogen status can be raised. With cultivation, however, nitrogen level again falls down to the normal. By the addition of organic matter or covering with grass, the nitrogen status is raised both due to fixation of atmospheric nitrogen and to its conservation in soils.

There is, however, one advantage of the high intensity of light and heat falling on tropical soils. The available nitrogen, *i.e.* the sum of ammoniacal and nitric nitrogen, is much greater in tropical soils than in temperate ones because of the greater facility of oxidation of organic and nitrogenous substances in the former. Thus, while the available nitrogen in the tropical soils, as observed by us, is about 10 to 30% of the total nitrogen (0.04-0.05%), the amount of available nitrogen in temperate country soils is only 1 to 2% of the total nitrogen (0.1-0.2%) persent in the soil. This, perhaps, explains why a better crop can be grown in the unmanured fields in tropical countries than those of the temperate countries under comparable conditions. Assuming that an acre of normal soils weighs, up to 9 inches depth, 1000 tons, the amount of total nitrogen in European soils will be about 2250 lbs. per acre, while in Indian soils it is about 1125 lbs. But the amount of available nitrogen per acre in European soils may be 22.5 to 45 lbs., whilst in Indian soils it is about 112.5 lbs. The concentration of the dissolved nitrate and ammonium salts in the soil solutions in tropics is

greater than that in temperate climates, and hence, absorption of these nitrogenous compounds by plant roots is much greater in a short time than in temperate climates. By adding molasses or easily oxidisable carbonaceous substances like cowdung, hay etc. to the soil, the nitrogen status is improved by nitrogen fixation. Along with improvement in the nitrogen status of soil, the available nitrogen also increases, thus making the soil more fertile. From our experiments with numerous molassed fields in Allahabad and elsewhere, we have observed that after a month or 5 weeks of the addition of molasses, not only the total nitrogen content of the soil is the highest but also the nitric and ammoniacal nitrogen content of the soil is largest ; and that is the time when the crop has to be sown in the field.

In my Address of 1935 (pp. 29-30) the probable mechanism of nitrogen fixation has been discussed. It has been stated that glucose can form pyruvic acid and hydrogen under anaerobic condition according to the following equation

$$C_6H_{12}O_6 = 2CH_3.CO.COOH + 2\ H_2 + 12\ \text{cal.}$$

In presence of nitrogen of the atmosphere and on the soil surface, the hydrogen obtained from decomposition of glucose can form ammonia according to the equation

$$N_2 + 3H_2 = 2NH_3 + 24\ \text{cal.}$$

In presence of oxygen, as under ordinary conditions, it is difficult for the production of hydrogen from glucose. It was also stated in the same Address that for obtaining the hydrogen (atomic) required for ammonia formation, the following reaction has to take place

$$H_2O + 112\ \text{cal.} = H + OH.$$

In other words, for obtaining a mol. of NH_3 according to the above equation, 336 cals. are needed. In the photosynthesis also, the breaking up of water into H and OH may be the chief reaction involved. Hence, the chief reaction involved in nitrogen fixation may be the same breaking up of a water molecule. The energy required for this breaking up of water is derived from the oxidation of energy materials needed for nitrogen fixation.

Marked Loss of Nitrogen in Nitrification

Crops have to depend a good deal on humus of the soil or farmyard manure. Moreover, physiologists have always emphasised that the body protein is preserved by both, carbohydrates and fats. We have carried out a very large number of experiments in the nitrification of different nitrogenous compounds in presence or absence of carbohydrates, lignins, celluloses, leaves, straw, etc. and found that in the process of nitrification, which is accelerated by absorption of light, there is more loss of nitrogen in the gaseous state than the amounts of nitrates formed, and nitrification and loss of nitrogen are markedly retarded by organic matter including coal. Hence, we have been emphasising since 1936 that a mixture of artificial nitrogen and organic matter should produce a better crop than artificials alone because the mixture contains more nitrogen in the process of nitrification than when the artificial is added alone. This will be clear from the following experimental observations.

TABLE VIII

	Substance mixed with soil.	Period of exposure. (Months).	%Loss of nitrogen.		%Loss of N in unit time per month.	
			Light.	Dark.	Light.	Dark.
1	Ammonium sulphate	½	55.5	43.2	27.8	21.5
2	,, phosphate	2	67.5	58 4	33.8	29.2
3	,, nitrate	2	28.9	21.0	14.5	10.5
4	,, tartrate	2	47.6	38.3	23.8	19.2
5	,, oxalate	2	36.6	28.6	18.3	14.3
6	,, citrate	4½	69.3	54.8	22.1	12.2
7	Urea	5½	47.4	35 1	10.5	7.8
8	Hippuric acid	4½	42.3	23.2	9.4	5.2
9	Gelatine	4½	40.1	23.2	8.9	5.2
10	Oil-cake	5½	35.9	29.0	6.5	5.3
11	Blood	6	54.1	48.7	1.0	8.1

TABLE IX

Retardation of nitrogen loss from soils with cowdung, green leaves and molasses.
277.2 kg. of nitrogen as $(NH_4)_2SO_4$ per acre of land used.

Condition.	NH_3—N.	NO_3—N.	Total N.	Total C.	Date of analysis.
Alone	0.028%	0.0026%	0.1167%	0.3721%	6-10-36
Cowdung	0.035	0 003	0.1272	0.5046	,,
Leaves	0.0304	0.0026	0.1137	0.3731	,,
Molasses	0.028	0.0031	0.1272	2.435	,,
Alone	0.0242	0.0039	0.100	0.3738	24-10-36
Cowdung	0.0324	0.0036	0.1082	0.5044	,,
Leaves	0.0236	0.0042	0.100	0.3792	,,
Molasses	0.0296	0.0039	0.1167	2.421	,,
Alone	0.0222	0.004	0.0875	0.3722	12-11-36
Cowdung	0.0304	0.0039	0.100	0.5010	,,
Leaves	0.0232	0.0049	0.0903	0.3812	,,
Molasses	0.0294	0.0054	0.1158	2.301	,,
Alone	0.0182	0.0061	0.0823	0.03768	26-11-36
Cowdung	0.0196	0.0062	0.0934	0.0498	,,
Leaves	0.0194	0.0073	0.0908	0.431	,,
Molasses	0.0248	0.006	0.1346	1.424	,,
Alone	0.0017	0.0064	0.0583	0.3712	30-12-36
Cowdung	0.0019	0.0078	0.0736	0.4882	,,
Leaves	0.0017	0.0082	0.0712	0.5122	,,
Molasses	0.004	0.0097	0.1273	0.9864	,,

The following results show marked loss of N in the nitrification of goat blood and its retardation by sodium citrate.

TABLE X

(500 g. soil + 100 c.c. blood).

Date.	State.	Total C in 100 g. of soil.	Total N per g. of soil.	NH_3–N per 100 g. of soil.	NO_3–N per 100 g. of soil.	Loss %.
10-9-47.	Exposed	1.357 g.	0.5653 g	0.0009 g.	0.0017 g	...
,,	Covered	1.357	0.5653	0.0009	0.0017	...
8-1-48.	Exposed	1.169	0.3067	0.0067	0.0280	45.7
,,	Covered	0.933	0.3125	0.0352	0.0243	44.2
11-3-48	Exposed	1.091	0.2594	0.0058	0.0289	54.1
,,	Covered	0.897	0.2900	0.0243	0.0259	48.7
30-7-48	Exposed	1.056	0.2131	0.0052	0.0321	62.5
,,	Covered	0.699	0.2678	0.0107	0.0282	52.6
15-9-48.	Exposed	0.802	0.1810	0.0044	0.0357	68.2
,,	Covered	0.501	0.2394	0.0091	0.0306	57.6

TABLE XI

(500 g. soil + 100 c.c. blood + 5 g. sodium citrate).

Date.	State.	Total C in 100 g. of soil.	Total N per g. of soil.	NH_3—N per 100 g. of soil.	NH_3—N per 100 g. of soil.	%Loss.
10—9—47	Exposed	1.647 g.	0.5831 g.	0.0009g.	0.0017 g.	...
,,	Covered	1.647	0.5831	0.0009	0.0017	...
8—1—48	Exposed	1.637	0.4682	0.0089	0.0242	19.7
,,	Covered	1.431	0.4699	0.0097	0.0234	19.4
11—3—48	Exposed	1 483	0.4320	0.0064	0.0357	25.9
,,	Covered	1.355	0.4500	0.0054	0.0289	22.8
30—7—48	Exposed	1.347	0.3899	0.0036	0.0308	33.1
,,	Covered	0.975	0.4285	0.0047	0.0311	26.5
15—9—48	Exposed	1 101	0.3421	0.0029	0.0426	41.4
,,	Covered	0.890	0.4048	0.0037	0.0342	30.6

The following results show the retardation of loss of nitrogen by the addition of lignites. Loss of nitrogen is greater in light than in dark.

TABLE XII

(NH_4NO_3+soil).

	Date.	Total C.	Total N	NH_3—N.	NO_3—N.	%Loss.
Light	20—9—50	0.464%	0.4413%	0.2015%	0.2032%	...
	20—10—50	0.446	0.2574	0.1031	0.1182	41.7
	20—12—50	0.412	0.2026	0.0773	0.0892	54.1
	20—2—51	0.378	0.1651	0.0596	0.0654	62.6
	20—4—51	0.342	0.1421	0.0492	0.0568	67.8
Dark	20—9—50	0.464	0.4413	0.2015	0.2032	...
	20—10—50	0.451	0.3080	0.1269	0.1450	30.2
	20—12—50	0.422	0.2613	0.1036	0.1216	40.8
	20—2—51	0.396	0.2362	0.0910	0.091	46.5
	20—4—51	0.368	0 2229	0.0882	0.0986	49.5

TABLE XII (contd.)

	Date.	Total C.	Total N.	NH_3—N.	NO_3—N.	% Loss.
			(Soil + urea).			
Light	20—9—50	0.634%	0.4412%	0.0014%	0.0029%	...
	20—10—50	0.610	0.3173	0.0256	0.0091	28.1
	20—12—50	0.566	0.2815	0.0201	0.0121	36.2
	20—2—51	0.511	0.2449	0.0101	0.0100	44.5
	20—4—51	0.468	0.2255	0.0090	0.0071	48.9
Dark	20—9—50	0.634	0.4412	0.0014	0.0029	...
	20—10—50	0.558	0.3582	0.0210	0.0150	18.8
	20—12—50	0.501	0.3218	0.0185	0.0152	27.1
	20—2—51	0.476	0.3016	0.0110	0.0100	31.6
	20—4—51	0.460	0.2701	0.0098	0.0084	38.7
			(Soil+lignite+NH_4NO_3).			
Light	2—7—51	4.166	0.4547	0.1895	0.1915	...
	2—8—51	4.008	0.2729	0.1091	0.0800	40.0
	2—11—51	3.694	0.2515	0.0820	0.0611	44.6
	2—12—51	3.380	0.2383	0.6620	0.0591	47.6
Dark	2—7—51	4.166	0.4547	0.1895	0.1915	...
	2—8—51	4.068	0.3170	0.1010	0.1216	30.3
	2—10—51	3.872	0.2979	0.0930	0.0988	34.5
	2—12—51	3.676	0.2829	0.0800	0.0731	37.6
			(Soil+lignite+urea).			
Light	2—7—51	4.336	0.4559	0.0029	0.0044	...
	2—8—51	4.170	0.3347	0.0291	0.0220	26.6
	2—10—51	3.838	0.2966	0.0156	0.0101	34.0
	2—12—51	3.506	0.2740	0.0099	0.0100	39.9
Dark	2—7—51	4.336	0.4559	0.0029	0.0044	...
	2—8—51	4.219	0.3801	0.0280	0.0299	16.6
	2—10—51	3.985	0.3432	0.0210	0.0156	24.7
	2—12—51	3.761	0.3146	0.0176	0.0138	31.0

Practical agriculturists have reported a marked loss of nitrogen in cultivation. Sir John Russell has recorded a loss of 60% of nitrogen added in the form of farmyard manure at the rate of 200 lbs. of nitrogen per acre per year in the Rothamsted fields. Dhar has recently calculated and found a similar loss from the field trials in Rothamsted using ammonium sulphate or sodium nitrate. Shutt in Canada and Lipman, Blair and others in U. S. A. reported a loss of 68 to 100 lbs. of nitrogen per acre per year. In his book ("Soil Conditions & Plant Growth", 1937) Russell stated that in bacterial nitrification of nitrogenous compound no loss of nitrogen takes place. But, recently, Dhar and Pant reported losses of 60% of nitrogen in the bacterial nitrification of different nitrogenous compounds.

NITROGEN LOSS—A GENERAL PHENOMENON IN AMMONIA OXIDATION

For a number of years we have been carrying on extensive research work on the slow oxidation of ammonium salts and other nitrogenous compounds by air in presence of soil etc. We have observed that with ammonium sulphate and other ammonium salts, there is a loss of about 60% of nitrogen more in light than in the dark. We

have ascribed this loss in the nitrification of nitrogenous compounds in soil at ordinary temperature to the formation and decomposition of the unstable substance, ammonium nitrite.

On the other hand, in the industrial preparation of nitric acid from ammonia Kuhlmann in 1839 observed that platinum was an excellent catalyst. Ostwald pushed this research further and reported that it was advisable to pass a mixture of air and ammonia as rapidly as possible over the catalyst, and that for each catalyst, there was a definite velocity at which a maximum yield was obtained. With platinum the yield of nitric acid from ammonia is 95% and this is the present industrial method. Germans have used other catalysts, but the yield of nitric acid is smaller than with platinum.

Ammonia burns in air under the influence of catalysts and forms, in the first instance, a white fog of nitrite and nitrate. At 300° reddish brown vapours appear and with increasing temperature more and more free nitrogen and steam are produced. It has been postulated that the following reactions take place between ammonia and oxygen :

1. $4NH_3 + 5O_2 = 4NO + 6H_2O + 215.6$ cal.
2. $2NH_3 + 3O_2 = 2HNO_3 + 2H_2O + 153.7$ cal.
3. $NH_3 + 2O_2 = HNO_3 + H_2O + 80.9$ cal.
4. $4NH_3 + 3O_2 = 2N_2 + 6H_2O + 302$ cal.
5. $4NH_3 + 7O_2 = 4NO_2 + 6H_2O + 269.5$ cal.
6. $4NH_3 + 6NO = 5N_2 + 6H_2O + 431.6$ cal.

According to Neumann and Rose 90% of oxidation products consist of nitrous acid when ammonia burns in air. In presence of oxygen 70 to 80% of nitric acid and 20 to 30% of nitrous acid are formed. From the very beginning of the oxidation reaction (4) liberation of free nitrogen takes place along with other reactions, as stated above. The extent to which it occurs increases with the rise of temperature. Reaction (4) also depends on the amounts of catalysts used and on the velocity of gaseous mixture. Reaction (6) is very important as it already takes place at a temperature at which the formation of nitric oxide commences. Reaction ($NH_4NO_2 = N_2 + 2H_2O + 718$ cal.) also gives rise to the formation of nitrogen gas. All the above reactions are exothermic and cause a rapid rise of temperature as soon as one or the other of them starts.

Ostwald attempted to increase the yield of nitric acid by the catalytic oxidation of ammonia by increasing the flow of gaseous mixture. The observations of Neumann show conclusively that the maximum yield of nitric acid by the catalytic oxidation of ammonia is obtained at a temperature of about 500°. At lower and higher temperatures more gaseous nitrogen is produced. With other catalysts the production of nitrogen gas is more pronounced. Hence, in industrial operations, even today, platinum is practically the only catalyst used.

The real factor for increasing the yield of nitric acid is to convert the whole of the ammonia into its oxidised products quickly, because, when ammonia is present, it reacts rapidly with the oxidised products, nitrous acid, nitrogen dioxide, and very likely, with nitric acid at a high temperature.

When ammonium salts, urea, uric acid, protein, oil-cakes etc. are added to soils, the first product formed is ammonia from protein which, in its turn, is converted into oxidised products like nitrite and nitrate. It is clear therefore that under these conditions, at ordinary temperature, the whole of the ammonia cannot be converted into oxidised products readily, and hence, ammonium ion, and occasionally free ammonia, have to co-exist with nitrite ion or nitrous acid, nitrate ion and/or nitric acid, and thus decomposition takes place with evolution of nitrogen gas. Evolution of nitrogen gas from nitrogenous compounds in soil or in surface has to be more pronounced than in the catalytic oxidation as in the industrial processes, because oxidation of ammonia at high temperatures in the catalytic process has a larger velocity than that at ordinary temperatures in soils or in presence of sand surfaces.

There is no doubt that the loss of the nitrogenous matter in soil is slowed down by addition of organic matter and thus crops can extract ammonium ion and nitrate under such conditions for a longer period, and hence, under ideal conditions, the recovery of nitrogen, which is believed to be 50% or less, can be improved by mixing ammonium salts with organic matter. But this loss can never be stopped, because the negative catalytic effect of carbohydrates and other substances decreases with the decrease in the concentration of such carbohydrates which are liable to be oxidised on cultivation.

Farmyard Manure or Straw mixed with Artificials produces more Crop

On the other hand, if the nitrogenous matter is associated with minerals like sodium salts, potassium salts, calcium salts, the process of ammonification and nitrification is likely to produce less nitrogen gas, because a part of the ammonium nitrate formed is likely to be converted into ammonium sulphate or chloride and alkali nitrites which are more stable. Our recent experiments show that addition of potassium chloride to ammonium salts or urea appreciably decreases the loss of nitrogen by nitrification.

The following data recently obtained at Rothamsted show clearly that both straw and cowdung, when added to artificials, produce beneficial results.

Table XIII

	Yield of potatoes in tons per acre.	
	No N, no K.	N, no K.
No farmyard manure ...	4.0	4.4
With „ „ ...	9.3	11.1
Response to farmyard manure ...	5.3	6.7

It is evident from the above table that the yield of potatoes should have been 9.7 tons (9.3 tons due to farmyard manure and 0.4 ton due to artificial nitrogen), but the total yield is 11.1 tons. These results show that addition of farmyard manure to artificial nitrogen produces an enhanced effect of the added mineral nitrogen.

Crowther and Yates (*Emp. J. Expt. Agrl.*, 1941, **9**, 77) have reported as follows:—

"The response to phosphate and potash are substantially reduced when dung is applied; but crops are equally responsive to inorganic nitrogen on dunged and undunged land. With potatoes there are positive interactions between nitrogen and phosphate and between phosphate and potash, but there is little interaction between nitrogen and potash".

One thing is perfectly clear from these results obtained with different manures that in presence of dung response to potash or phosphate is half or less than that in the absence of dung, whilst with nitrogen the position is entirely different, and response to unit weight of nitrogen does not fall off as the total dressing is increased by addition of 28 lbs. of nitrogen as ammonium sulphate. The curve of response of artificial nitrogen in presence of dung is much steeper than that with potash or phosphate in presence of dung. This conclusion can only be explained satisfactorily from the viewpoint that organic matter present in the farmyard manure enhances the value of artificial nitrogen.

Moreover, the following experiments on potatoes, carried out at Rothamsted for a number of years in which a given dressing of straw and nitrogen was ploughed into the soil, are compared with the same quantity of straw rotted with the same quantity of nitrogen in a compost heap and then applied to the land. The ploughed-in straw has always a better yield than the compost as shown below.

TABLE XIV

12 years (1904-45).

	Year of applying straw.		Year after application.	
	Compost.	Straw ploughed-in.	Compost.	Straw ploughed-in.
Potatoes (tons per acre)	7.86	9.40	7.40	7.97
Sugarbeet, sugar (cwt per acre)	37.0	41.2	36.4	38.2
Barley, grain (,, ,,)	27.7	31.2	26.5	27.9

The above experimental results obtained at the famous Research Station of Rothamsted are strong support of the conclusion drawn in 1935 by me from our experiments that a mixture of ammonium sulphate and organic matter is a better fertilizer than ammonium sulphate alone. Similar results have also been obtained in U.S.A. In "Commercial Fertilizers" by G. H. Collings (1947, p. 436), it has been stated:—

"Many agricultural experiment stations have shown that the incorporation of green manures in the soil, in addition to a complete fertilizer, results in an increase in the yields of succeeding crops. Most soils are benefitted by the addition of suitable organic matter regardless of the quantity they contain. Johnson (1924) at the Virginia Truck Experiment Station, found that when one ton of a 6-4-8 fertilizer was applied, the average yield of potatoes was 155 bushels, but when a green manure crop was turned

under previous to the addition of the fertilizer, the averge yield was raised to 232 bushels". Again, on page 213, it has been stated : "When virgin soil is put under cultivation, a rapid decay and nitrification of the organic matter is induced, and the resulting carbonic and nitric acids materially aid in making available the insoluble phosphates of the soil. As supply of organic matter decreases so do the quantities of carbonic and nitric acid and in turn the quantity of available phosphoric acid. This natural process has played an important role in increasing the demand for superphosphates as well as for the potashes in the older populated areas".

F. E. Bear and B. L. Prince (New Jersey Agricultural Experiment Station Bulletin, No. 757, June, 1951) have reported as follows :—

"When crop residues were returned to the soil, the yield increase in terms of total produce of snap beans and carrots averaged 12%. A rye cover crop added 7½%, and 2½ tons of cornstalks another 14%. Thus, for organic matter additions totalling a little more than 5 tons an acre a year, the yield increase was 33½%".

"The best organic matter system under continuous culture raised crop yields to 39% . This involved crop residues cover crops and soy beans for mulching every second year. The best 5-crop rotation programs raised average yields more than 60%. These involved letting the land grow up to weeds, using winter grains and deep-rooted legumes, growing sweet corns and soy beans or employing similar procedure two years out of every four".

Compositing of Organic Matter leads to Loss of Nitrogen

In a recent paper, Dhar and Ghildyal (*Proc Nat. Acad. Sci.*, 1951, **20**, 1) have described an elegant method of composting wheat straw by mixing it with 1/8th of its weight of soil without any other artificial fertilizer. In this composting process there is an appreciable fixation of nitrogen, whilst other workers have obtained marked loss of nitrogen in compositing, as is shown in Table XV.

In sunhemp composts, when C:N ratio came down from 22.2 to 12.37, about 18% of the nitrogen was lost in aerobic compost, and when it reached 11.19 in extra aerobic compost, 42.35% nitrogen was lost in the same period. Jayaraman (1914) has also observed large proportions of losses with low C:N ratio and gains with high C:N ratio (vide Table XVI).

Considerable amounts of nitrogen are similarly lost when substances with low C:N ratio, like dried blood, cotton seed etc., undergo decomposition.

C.N. Acharya and his co-workers have obtained a loss of ¼ of nitrogen in 20 weeks of composting in a mixed organic refuse, C:N ratio being 32. As loss of nitrogen is heavy even in the Adco process, it is not a profitable method, and that is why it has not been generally adopted.

Dhar and Ghildyal added 17.5 g. of nitrogen as ammonium sulphate to wheat straw containing 25 g. of nitrogen, and even after 60 days 4 g. of nitrogen were lost, whilst after 200 days 10 g. were lost in light. When 35 g. of nitrogen as ammonium sulphate were added, the loss in 60 days was about 11.5 g. and in 200 days the loss amounted to 23 g. in light. In the dark the loss of nitrogen was in general less. It is clear that 55% and 66% of the added nitrogen were lost in the process of composting by adding ammonium sulphate.

3—1811P—5

TABLE XV

Decomposition of organic constituents during composting in g. per 100 g. of original material.

	Oat straw Inorg salts.			Oat straw Clover hay.			Cow manure.			Peat Timothy hay.			Cornstalks Inorg salts.			Leaves Timothy hay.		
	At start.	At end (110 days)	Loss or gain (%).	At start.	At end (110 days).	Loss or gain (%).	At start.	At end (110 days).	Loss or gain (%).	At start.	At end (110 days).	Loss or gain (%).	At start.	At end (110 days).	Loss or gain (%).	At start.	At end (110 days).	Loss or gain (%).
Total material	100	44	56	100	34	66	100	47	53	100	62	38	100	49	51	100	38	62
Hemicelluloses	16.5	5.55	−66	15.5	4.17	−73	15.7	2.82	−82	10.5	4.17	−60	20.0	3.69	−81	13.0	3.48	−73
Cellulose	33.0	4.19	−87	30.8	5.10	−83	18.9	1.60	−92	7.8	0.88	−90	27.4	3.94	−86	15.7	1.82	−88
Lignin	13.4	11.55	−14	13.6	8.00	−41	20.8	11.00	−47	38.0	22.40	−38	9.5	10.12	+7	25.1	11.30	−55
Ash	5.5	9.65	+75	5.77	5.77	+4	17.3	17.38	0	10.7	12.91	+21	6.3	14.05	−124	10.2	10.50	+3
Total N	1.36	1.03	−24	1.24	0.85	−26	2.16	1.44	−33	2.38	2.00	−13	1.28	1.09	−15	1.13	0.70	−38
Total N	...	2.34	...	...	2.50	...	...	3.07	...	...	3.22	...	...	2.23	...	...	1.84	...

TABLE XVI

Material.	Initial C :N ratio.	Initial N lost.
1. Dadap leaves	12.3	50.60%
2. Tea	19.1	13.00
3. Grevillea	36 7	2.3 (gain).

In Drouineau and Lefevre experiments (*Ann. Agric.*, 1949, p. 328) the loss was heavier as the nitrogen present in the grape residues was also lost. In Dhar and Ghildyal's experiments with straw containing 0.704% nitrogen and C:N ratio of 55, there was appreciable fixation of nitrogen in composting with small quantities of soil. With sunhemp containing 1.45% nitrogen and C:N ratio of 22.3, there was a loss of nitrogen in the composting process. When straw and sunhemp were added to soil and allowed to undergo slow oxidation, there was nitrogen fixation. Hence, composting of plant materials is certainly wasteful from the nitrogen point of view. The Rothamsted results with potatoes show that when straw along with ammonium sulphate is added to soil, the crop yield is better than when the same mixture is added after composting. This is likely to be due to the fact that more nitrogen is lost in composting than in direct addition to the soil. It seems that in composting the nitrogenous matter is in a more concentrated form than when the mixture is added direct to the soil where it is diluted, and hence the concentration of ammonium nitrite formed is greater than that in soil. We have definitely established that the greater the concentration of ammonium nitrite, the greater is its percentage of decomposition. Moreover, acidity increases decomposition and that is why in temperate country soils the loss of nitrogen is as prominent as in hot country soils.

DEEP CULTIVATION CAUSES GREATER LOSS OF NITROGEN

It seems that shallow or deep ploughing is unnecessary when the soil is rich in organic matter. If the soils are porous, due to their richness in organic matter, the plant roots can easily penetrate without the soil being ploughed. Nobody has emphasised so far the fact that more nitrogen is lost from rich soils on cultivation than is taken up by crops. Hence, when a rich soil is ploughed, the fertility is lost not due to exhaustion by crop, but due to the formation and decomposition of the unstable substance, ammonium nitrite, from the soil proteins and other nitrogenous compounds. If the soil is rich in organic matter it will also be rich in nitrogenous compounds, and if seeded with wheat or oat, there will be plenty of available nitrogen in the soil up to a depth of 3 to 4 inches. If the soil is dug up to 8 inches, more nitrate may be available to the crop, but as the soil is rich already in its nitrate contents in the first 3 or 4 inches from the top, the additional nitrate available in the next 4 or 5 inches may not help the crop. The net effect will be the same crop production and more loss of nitrogen from the soil when ploughed up to 8 or 10 inches. Recent experiments carried out at Rothamsted show that, in many cases, crop production is the same whether the ploughing is 4 or 8 inches deep.

Even in India the tractor is breaking our lands deep. This may be suitable for growing exhausting crops like sugarcane without manure or fertilizers, but soil exhaustion is inevitable. It is clear therefore that deep cultivation, specially in rich soils,

is sure to cause exhaustion more rapidly than shallow cultivation. The problem is to avoid deep cultivation as much as possible as it involves marked loss of nitrogen in the gaseous state due to formation and decomposition of the unstable substance, ammonium nitrite.

Phosphates enhance markedly Nitrogen Fixation in Soils

Recently, we have shown that in presence of phosphates in sand as medium, an efficiency of 200 mg. of nitrogen fixed per g. of carbon oxidised has been obtained in light. In soils fairly rich in calcium and phosphate, nitrogen fixation of the order of 150 to 75 mg. in light in unsterile conditions and of the order of 84 to 65 mg. under sterile condition has been observed with wheat straw as recorded in the following tables. In the dark the values vary from 80 to 35 in unsterile condition and from 43 to 32 under sterile conditions. Hence there is a marked light effect in this type of nitrogen fixation.

Table XVII

Analysis of soil.		Analysis of wheat straw.	
Total carbon	1.598%	Total carbon	39.17%
Total nitrogen	0.208	Total nitrogen	0.6375
CaO	3.402	C : N ratio	61.4
P_2O_5	0.418	Moisture	4.316
MgO	0.405	Loss on ignition	91.49
K_2O	1.139	Ash	8.51
SiO_2	75.412	SiO_2	7.05
Loss on ignition	6.86	CaO	0.2884
		MgO	0.0980
		K_2O	0.7025
		P_2O_5	0.0619

Table XVIII

Experiments carried in a 60 watt bulb light.

Period of exposure.		Total carbon.	Carbon oxidised.	Total nitrogen.	Change in nitrogen.	Efficiency.
		200 g. soil+2 g. wheat straw. Light..				
1 (a).	0	1.9673%	0	0.2117%	0	.....
	80	0.9734	0.9939%	0.3606	0.1489%	149.8
			Dark.			
(b).	0	1.9673	0	0.2117	0	.....
	80	1.0300	0.9373	0.3108	0.0991	105.7
		200 g. soil+2 g. wheat straw + 2.521 g. Na_2HPO_4. $12H_2O$. Light.				
2 (a).	0	1.9510	0	0.2097	0	.....
	80	0.7466	1.2044	0.3866	0.1769	146.8
				"Dark".		
(b).	0	1.9510	0	0.2097	0	.....
	80	0.8024	1.1486	0.2925	0.0828	72.1
		200 g. soil+2 g. wheat straw+0.5 g $CaCO_3$ "Light".				
3 (a).	0	1.9664	0	0.2112	0	.....
	80	0.6572	1.3092	0.4010	0.1898	144 9
				"Dark".		
(b).	0	1.9664	0	0.2112	0	.....
	80	0.7966	1.1698	0.3271	0.1159	98.5

Sir A. D. Hall (" An Account of the Rothamsted Experiments", 1905) has stated as follows regarding the Rothamsted soils:

"There is an abundant stock of potash. A complete mineral analysis in which the Broadbalk soil was completely broken up by H_2F_2 yielded 2.26% of potash, four times the amount that can be extracted by long digestion with HCl. In phosphoric acid the soil is by no means so rich. The unmanured plots contained in 1905 less than 0.1%; the highest limit reached in the heavily manured plots was 0.25%. Under ordinary farming conditions the soil shows no particular need of phosphoric acid as do many clay soils".

Sir John Russell (" Soil Conditions & Plant Growth ", 1932, p. 479) has stated as follows:-

"Phosphorus is peculiar in that some soils are notably deficient in it: these occur commonly in Australia and South Africa, but also in the United Kingdom: in consequence the vegetation is affected and the grazing animals suffer serious phosphate starvation." Again on page 554 he has stated:" Phosphate exhaustion was the most serious occurrence because there was no way of meeting it, and as the original supplies were not, as a rule, very great, it must have become acute by the end of the eighteenth century in England, for remarkable improvements were, and still are, effected all over country by adding phosphates. Then began a process, which has gone on to an increasing extent ever since, of ransacking the whole world for phosphates; at first the search was for bones, even the old battle-fields not being spared, if we may believe some of the accounts that have come down; later on (in 1842) Henslow discovered large deposits of mineral phosphates to which more and more attention has been paid. Phosphate supplies may yet become the factor that will determine the course of history".

Similarly, F. G. Clarke (The Data of Geochemistry Deptt. Int. U. S. Geological Survey Bulletin 770, 1924) has reported that the potash content of humid soils of U.S.A. is 0.216% and in arid soils it is 0 729%, whilst the P_2O_5 content is 0.113% and 0.117% respectively.

Table XIX shows that in many American soils the phosphate (P_2O_5) is often smaller than the total nitrogen content, and is much smaller than the potash (K_2O) content.

In unmanured Danish soil also the P_2O_5 content is low, *i.e.*, 0.052%, and when farmyard manure is added, it goes up to 0.064% (Forsog med Staldgodrungog Ved Karsten Iverson og K. Darph—Peterson).

Thus it appears that in most countries the normal soil contains 0.1% or less P_2O_5, and thus deficient in phosphate.

Our experimental results show that when nitrogen fixation experiments are carried on with soils having a P_2O_5 content of 0.075%, the efficiency of nitrogen fixation per gram of carbon oxidised is much smaller than with soils having a phosphate content varying from 0.33 to 0.41%, both under sterile and unsterile conditions with different energy materials. Hence, we have come to the important conclusion that the nitrogen status of a soil is intimately connected with its phosphorus status and that phosphates can not only act as food for plants, micro-organisms and buffers in controlling the p_H of soil but can help in the fixation of atmospheric nitrogen by the oxidation of energy materials.

TABLE XIX

[From "Farm Soils, Their Management and Fertilization" by E.L. Worthen, 1948, Table III, p. 61].

Description of soil.	Location.	Pounds of total plant food in 2,000,000 pounds of soil. Nitrogen.	Phosphoric acid P_2O_5.	Potash K_2O.	Lime. CaO.
Limestone valley	Pennsylvania	2,400	2,300	69,600	...
Residual (shale)	West Virginia	2,750	1,624	26,400	...
Glacial (limestone)	New York	4,800	3,220	40,440	2,520
Glacial (shale)	New York	5,200	2,732	44,616	259
Coastal plain	New Jersey	1,300	3,119	18,836	2,560
Maryland barrens	Maryland	...	414	2,400	812
Piedemont plateau	North Carolina	790	1,288	55,440	4,452
Coastal plain sand	South Carolina	759	867	11,992	...
Timbered glacial soil	Ohio	1,860	2,116	4,756	5,180
Gray prairie	Illinois	2,777	1,868	30,798	5,820
Brown prairie average	Iowa	5,458	3,209	34,490	...
Peat (1000,000 lbs.)	Wisconsin	33,900	3,611	5,820	...
Upland Brazos Co.	Texas	1,620	695	15,518	6,201
Gray timber soil	Missouri	2,800	2,530	41,821	18,400
Prairie	Kansas	2,800	1,686	19,716	10,800
Residual limestone	Kentucky	...	1,895	32,063	11,918
Black prairie (glacial)	North Dakota	6,335	3,050	31,100	...

In culture experiments on nitrogen fixation by Azatobacter, A. Koch and S. Seydal (*Centrbl. Bakt.*, II, **31**, 570, 1912) have reported that 70 to 80 mg. of nitrogen are fixed by Azotobacter per gram of glucose oxidised on the second and third day of growth, and only 5 to 8 mg. on the eighth day. Although the total amount of nitrogen fixed during the first 5 days is small, the process is economical only in the beginning. Similarly, J. G. Lipman (N. J. Agr. Expt. Sta. Am. Rept. 24, 217, 1933) has obtained the following results in cultures :

Mannitol (%)	0.1	0.2	0.5	1.0	1.5
Nitrogen fixed (in mg. per g. mannitol)	10.5	8.3	6.4	4.68	3.22

These results on nitrogen fixation by Azotobacter in culture media are certainly not as efficient as those obtained by us with carbohydrates and celluloses.

NITROGEN FIXATION IN SOILS MORE CHEMICAL THAN BIOLOGICAL PROCESS

In recent years Dr. H. L. Jensen in Lyngby (Denmark), Dr. Stockli in Zürich and Prof. Lemoigne in Paris have emphasised that Azotobacter does not play a big role in

increasing the nitrogen status of soils. From our experiments in sterile and unsterile conditions, it is clear that bacterial action is not as prominent as surface reactions in soils. Hence, the existence or non-existence of bacteria should not materially affect the nitrogen status of the soil as the efficiency of nitrogen fixation is of the same order, both under sterile and unsterile conditions. It appears that the microbiological action is vigorous either in culture media containing easily decomposable energy materials or in sewage or fermentation processes. On the other hand, if the energy materials to be utilised by the micro-organisms and the microbial population are diluted or mixed up with large quantities of inert and difficultly soluble materials like sand, soil, iron oxide, zinc oxide, nickel oxide etc., the intensity of the microbial action decreases. In most experiments for the isolation or detection of bacteria, the conditions are rendered highly suitable for their rapid multiplication by supplying energy materials for their growth and activity and mineral requirements. That is why Azotobacter or Clostridia have been found to multiply and fix nitrogen in tubes or flasks; but under natural conditions in fields, the amount of nitrogen fixed by the addition of energy materials has not been accurately determined. In culture experiments in flasks containing energy materials like glucose, mannitol etc. and minerals like lime, potash, phosphate, iron etc., they are readily available to the bacteria in a state of solution. On the other hand, even if 10 tons of molasses or straw are added per acre of land, it is diluted with 99% of its weight of inert silicates, iron oxides, humus etc. present in the soil, and hence the chance of bacteria and the food materials to come in contact with each other appears to be much less than in culture media in flasks. It is clear therefore that the chemical changes involved in bacterial reactions with energy materials are bound to be slower in soils than in culture media, and that is why on adding organic matter to the soil, a longer time interval is necessary before the oxidation and nitrogen fixation are observed than in culture media. This explains the failure of H. K. Jensen (*Proc. Linn. Soc. N. S. W.*, 1940, 651) to observe nitrogen fixation in many samples of New South Wales soils during an incubation period of 30 days when mixed with glucose as energy material. It appears that the nitrogen fixation in 30 days in soils is much less than in culture media containing glucose. In cold countries the soil nitrogen fixation is slower than in warm countries. Moreover, in cold countries the nitrogen status of soils is higher than in warm countries, and the apparent nitrogen fixation on adding energy materials is less prominent than in hot countries.

By the application of three doses of Neem leaf (*Melia Azadiracta, Linna*) to field soils containing 0.0386% total nitrogen, the nitrogen status was raised to 0.1025% by nitrogen fixation and the C: N ratio of the soil became 10. Similarly, by adding cowdung three times, the nitrogen status could be raised to 0.2% in 3 years from 0.0386%. Moreover, on applying municipal rubbish, the nitrogen status was raised to 0.25% from 0.039%, and bumper crops were grown on this land. The classical Rothamsted experiments show that, by adding farmyard manure at the rate of 14 tons per acre per year and growing a crop every year on this land, the nitrogen status was raised to 0.256% from 0.122%. Similar experiments carried out with farmyard manure in Denmark showed the following increase of the soil nitrogen.

TABLE XX

			Increase with farmyard manure.	Increase with artificial fertilizers.
Sandy soil	...	0.066% N	30.3%	15.1%
Lyngby soil	...	0.146	15.7	3.4
Askov loam	...	0.106	22.6	11.3
Lundgaard	...	0.106	10.3	3.7
Tylstrup	...	0.118	6.1	1.6

Moreover, we have been able to show definitely that marked nitrogen fixation takes place under completely sterile conditions, both in light and dark. Hence, it appears that the nitrogen status of all soils of all countries not under legumes appears to be chiefly due to surface- and photo-oxidation of all kinds of energy materials and consequent nitrogen fixation aided by phosphates.

MECHANISM OF NITROGEN FIXATION

In explaining the photosynthesis in plants, Dhar postulated in 1933 that the important photochemical reaction was the decomposition of water by absorption of light according to the following equation :

$$H_2O + 112 \text{ cal.} = H + OH.$$

The hydrogen atom, thus formed, reduces the carbonic acid adsorbed on the leaves forming formaldehyde. In recent years this view of the mechanism of photosynthesis has been supported by the use of carbonic acid containing isotopic carbon. In explaining the nitrogen fixation the best mechanism seems to be the same, *i. e.* the decomposition of water into H and OH by absorption of energy obtained from the oxidation of carbohydrates, celluloses, lignin, fats, coals etc. Moreover, in presence of light, the light energy, whether from the sun or artificial source, is absorbed by the system and utilised in increasing the nitrogen fixation. In symbiotic nitrogen fixation also the same mechanism may be applicable.

For fixing 14 g. of nitrogen and forming ammonia by the interaction of molecular nitrogen and atomic hydrogen obtained by the decomposition of water, as stated above, 336Kcal. are needed. Hence, from the oxidation of a gram mol. of glucose according to the equation

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 676 \text{ cal.},$$

$14 \times 676/336$ g. of nitrogen can be fixed under ideal conditions. In other words, 0.39 gram of nitrogen should be fixed per gram of carbon oxidised. In many of our experiments in soils, fairly rich in calcium and phosphate, a nitrogen fixation of 100 mg. with sugar candy and about 150 mg. with wheat straw, has been observed per gram of carbon oxidised. Hence, the efficiency of this type of nitrogen fixation in soils is $150/390 \times 100 = 38\%$ with straw in artificial light, and with sugar candy $100/390 \times 100 = 25\%$. In sterile conditions the efficiency in light is 21% with wheat straw, while in dark this efficiency is 10%.

It is well known that in absence of moisture nitrogen fixation in soils does not take place, because water plays a prominent part as postulated in the mechanism of nitrogen fixation proposed above. The ammonia can readily be oxidised to nitrates in soils or nodules. Dhar and Mukherji (*J. Indian Chem. Soc.*, 1934, **11**, 727) have obtained amino-acids readily by the action of nitrates on carbohydrates in presence of light using titania as a photo-catalyst. In symbiotic nitrogen fixation the energy required is supplied by the oxidation of carbohydrates photosynthesised by the host plant, and nitrogen fixation takes place chiefly in the nodules. In this process the possibility of the loss of nitrogen by the formation and decomposition of ammonium nitrate appears to be less than in our experiments where the facility for the oxidation of proteins, amino-acids and ammonium salts obtained by fixation is greater than that in nodules. This is certainly one of the reasons why symbiotic nitrogen fixation frequently appears to be more efficient than non-symbiotic fixation.

Ammonia is readily detected in nitrogen fixation by Azotobacter or Clostridia in culture media. In our experiments on nitrogen fixation in sand or soil with soluble carbohydrates or glycerol as energy materials, there is a marked increase of ammoniacal nitrogen in a short time. Moreover, using 'Sanai' (sunhemp) as energy material in nitrogen fixation, we have frequently observed a pronounced smell of free ammonia when the nodules are rubbed under fingers.

It is well known that in symbiotic or non-symbiotic nitrogen fixation there is hardly any increase of nitrogen in presence of small amounts of ammonium salts or nitrate. Similarly, in our experiments the nitrogen fixation falls off on the addition of ammonium salts. This is due to the fact that the fixation is opposed by the phenomenon of loss of nitrogen involved in nitrification caused by the formation and decomposition of the unstable substance, ammonium nitrite. This loss is retarded by the presence of carbohydrates which act as negative catalysts in nitrification. It appears that the fundamental chemical changes involved in all types of nitrogen fixations are identical. In symbiotic fixation the Rhizobia, containing haemoglobin, may be able to oxidise the carbohydrates synthesised by the host plant very rapidly creating an efficient nitrogen fixation process which certainly requires an influx of energy. Also phosphates and calcium carbonate seem to increase the efficiency of nitrogen fixation in all types of nitrogen fixations in which ammonia formation is an important step.

Efficiency of Symbiotic Nitrogen Fixation

P. W. Wilson ("Biochemistry of Nitrogen Fixation", Madison, 1940) has reported that in nitrogen fixation by red clover, using sucrose in sand culture, a nitrogen fixation of 1 gram has been obtained per 25 grams of dry plant material. Assuming that 40% of this plant material is carbon, the symbiotic nitrogen fixation by red clover becomes 100 mg. per gram of carbon oxidised in symbiosis. It is very interesting therefore that the efficiency of symbiotic nitrogen fixation, which has always been considered to be 10 times greater than that of non-symbiotic fixation, may be even smaller than the efficiency obtained in light in our experiments. In the manuring of fields with legumes Weir has stated that under normal condi-

tions 1 cwt. (*i.e.* 112 lbs.) of nitrogen is added per acre. but usually the amount may be as small as 40-60 lbs. On the other hand, when 5-10 tons of leaves or straw are added to the soil, specially in tropical countries, the nitrogen addition may be much greater than with legumes. Moreover, legumes cannot be grown year after year in the same soil. Also, legumes make the soil certainly more acidic than straw or plant leaf and are known to be an acidic manure. It seems therefore that there is very little to choose between legume manuring and carbohydrate manuring, specially in lime- and phosphate-rich soils. The carbohydrate manuring or lignite manuring is specially suitable in soil containing low total nitrogen and neutral type. Recent experiments carried on in Nigeria with 1 ton of farmyard manure per acre have produced excellent results in crop production because of the low nitrogen content of the soil. This is in support of our observations and conclusions. In cold countries carbohydrate and lignite manuring should be quite suitable in presence of calcium carbonate or calcium phosphate, as our experiments show that in the presence of calcium carbonate the oxidation of both, carbohydrate and straw, is intensified in nitrogen fixation.

Failure of Chemical Technology to cope with World Food Production

As the industrial methods of nitrogen fixation are not very efficient, the price of artificially fixed nitrogen has remained high. The world production of this commodity in 1937 was 3.54 million tons. Even today the total production may not exceed 4 or 5 million tons. For producing 600 million tons of cereals and 400 million tons of good food material (these are the amounts produced at present in the world) no less than 150 million tons of combined nitrogen are needed. Hence, synthetic nitrogen has failed to cope with world food production. It is no wonder, therefore, that in the 1949 Meeting of the British Association, it was concluded that only 3% of the world food production has to be attributed to synthetic nitrogen and 97% of the food is derived from the nitrogen of the soil humus. It is clear therefore that nitrogen fixation in soil has to be improved markedly not only by growing legumes, as is being done in all the advanced countries, but nitrogen fixation in presence of sunlight by addition of all kinds of energy materials mixed with phosphates to the soil has to be intensified, bacause organic matter not only supplies the minerals and nitrogen it contains but also fixes atmospheric nitrogen readily in the soil and protects the soil nitrogen from loss in the gaseous state.

It is interesting to note that the immortal chemist Lavoisier was much impressed by the value of grass in improvement. Recently, T. L. Lyon and H. O. Buckman ("The Nature and Properties of Soils", New York, 1943, p. 393) at Ithaca, have reported that the soil under a grass ley free from legumes gained 40 lbs. of nitrogen per acre per year. Similarly, J. W. White, F. J. Holben and A. C. Richer (*J. Amer. Soc. Agron.*, 1945, **37**, 21) in Pennsylvania observed that the soil under a grass ley not containing legumes, gained 15-30 lbs. of nitrogen annually, even though they were unable to isolate Azotobacter from the soil. Similar gains in nitrogen of grass lands have been reported in Rothamsted.

It appears that the carbonaceous matters added to the soil as plant parts or farmyard manure are oxidised thermally and photochemically on the soil surface and lead to nitrogen fixation even in absence of leguminous plants or Azotobacter.

In a recent paper, A. Demolon (*Rev. Gen. Botanique*, 1951, **58**, 489) has concluded that the symbiotic fixation of nitrogen has to be enhanced by controlling and improving the processes of this fixation. We are trying to intensify the growth of leguminous plants by adding straw, leaves, farmyard manure etc. to the soil before growing these plants.

The Key Element of Cereal

It has been reported by Sir John Russell that an average yield of 12 bushels (1 bushel=30 seers or killograms) of wheat is obtained per acre from an unmanured land in Rothamsted, England; when phosphate and potash are added the yield goes up to 14 bushels, *i.e.* an increase of 15%; when 43 lbs. of nitrogen as ammonium sulphate or sodium nitrate are added along with phosphate and potash, the yield goes up to 23 bushels, *i.e.* an increase of 85%. With 129 lbs. of nitrogen per acre the yield of wheat becomes 36 bushels *i.e.* an increase of 200%. It is clear therefore that nitrogen is the key element in wheat production.

The average annual yield of wheat in different countries per acre at the end of the last century was as follows:—

Denmark	...	...	...	41.8	bushels
U. K.	...	...	...	29.1	
U. S. A.	...	...	...	12.0	
South Australia	...	...	...	7.0	

At that time the bulk of the world supply of wheat came from U. S. A. and that is why the world average was 12.7 bushels per acre. The higher yield in the European countries was chiefly due to the application of ammonium sulphate or sodium nitrate and other fertilizers.

Crookes' Prediction

In September 1898 Sir William Crookes delivered a memorable address before the members of the British Association at Bristol and he stated :

"I stated that under present conditions of heedless culture, a scarcity of wheat is within appreciable distance ; that wheat-growing land all over the world is becoming exhausted, and that at some future time—in my opinion not far distant—no available wheat land will be left. But I also pointed out that Nature's resources, properly utilised, are ample. I urged that, instead of being satisfied with an average yield of 12.7 bushels per acre, a moderate dressing of chemical manure would pull up the average to 20 bushels—thus postponing the day of dearth to so distant a period that we and our sons and grandsons may legitimately live without undue solicitude for the future".

Crookes also stated : "For years past attempts have been made to effect the fixation of atmospheric nitrogen and some of the processes have met with sufficient partial success to warrant experimentalists in pushing their trials still further, but I think that I am right in saying that no process has yet been brought to the notice of

scientific or commercial men which can be considered successful, either as regards cost or yield of products. The fixation of atmospheric nitrogen, therefore, is one of the great discoveries awaiting the ingenuity of chemists. It is certainly deeply important in its practical bearings on the future welfare and happiness of the civilised races of mankind" ...and "...unless we can class it among certainties to come, the great Caucasian race will cease to be foremost in the world, and will be squeezed out of existence by races to whom wheaten bread is not the staff of life".

Crookes also stated : "Starvation may be averted through the laboratory—the chemist will step in and postpone the day of famine".

Ostwald's Warning to Germans

The following observations of the great German chemist, W. Ostwald, in 1903 played a very important part in the development of the nitrogen industry:—

"Nebender Landwirtschaft hat aber an den chilenischen salpeter lagern noch ein andere instanz ein Lebensinteresse die Heersverwaltung ohne salpeter ist heute das best Heer nahezuwehrlos, denn alles schiess pulver, Vom ehriuurdigen schwarz pulver bis zu dem modernsten rauchlosen Material, wird direkt order indirekt aus salpeter hergestelit und Kann auf auf enderem wege nicht gewon nenwerden. Wenn heute ein Krieg Zwischen zwel grossen Machten ausbrachte von denen eine inder Lage ware, dei Ausfuhr des salpeters aus den weningen Hafen chiles zu verhindern so wurde sie ihren Gegner dadurch Kamfunfahig machen konnen, dass sie den Krieg solange fortsetzte, bis dieser seine Munition ver brancht hat".

Nitrogen Fixation Industry

It appears that Crookes thought of nitrogen fixation in terms of averting starvation and famine, whilst Ostwald talked of nitric acid required for explosives demanded in large quantities in warfare. It is interesting to note that the good of humanity by improving crop production did not appeal so much as the production of explosives, because the well-to-do people all over the world have plenty of food even today and do not bother so much about food production as earning money of which explosives are more profitable than nitrogenous compounds required for agriculture.

At present the nitrogen industry is as important as the steel and coal industry. The amounts of nitrogen produced just before the last war were as follows.

Table XXI

Nitrogen fixed in tons (1937).

Country.	Plants.	Quantity.	Country.	Plants.	Quantity.
Germany	13	13,65,850	Norway	4	1,21,000
Japan	21	4,90.132	Canada	3	1,02,000
U.S.A	10	2,92,510	Manchuria	1	40,000
England	2	2,32,870	Sweden	3	14,000
France	27	2,44,050	Switzerland	3	13,200
Belgium	10	2,17,980	Spain	2	8,000
Soviet Union	4	1,57,500	China	2	7,175
Italy	18	1,46,830	South Africa	1	5,740
Holland	3	1,36,630	Hungary	1	5,750
				Total	35,47,352

Our country has started a nitrogen fixation factory based on Haber-Bosch method in Sindri, Manbhum, Bihar, but the cost of production of fixed nitrogen is very high.

The amount of nitrogen necessary for the production of a single good cereal crop from an acre of soil is about 25-50 lbs. because this is the amount actually present in the rain and the hay. However, in the advanced foreign countries the actual amount of nitrogen supplied in the form of artificial manure is much less than the requisite amount.

TABLE XXII

Nitrogen used per acre of land under cultivation in 1937.

Country.					
Holland	24.79 lbs.	Norway	5.98 lbs.	Great Britain	2.49 lbs.
Belgium	28.55	Sweden	5.24	United States	1.36
Germany	15.65	France	4.00	Poland	0.73
Denmark	10.26	Italy	4.29	Hungary	0.15

At present the nitrogen added to field is increasing in Europe and America.

Only 3% of the world crop yield has been attributed to artificial nitrogenous manures in the 1949 British Association meeting and reported in "Nature" (1949 **164**, 579) as follows:—

"At present only some 3 per cent of the world food production can be attributed to the use of nitrogenous fertilizers. To raise this by 10 per cent, that is to say, 100 million tons, involves a fourfold increase in supplies of fixed nitrogen at an approximate capital cost of £1,50,00,00,000 (Rs. 2,100 crores). This would take a minimum of 15 years to achieve".

It is interesting to note that the Europeans who were controlling the destiny of the world lived chiefly on bread, meat and wines till the first world war,and naturally the Rothamsted Experimental Station which was started by Sir Bennett Lawes in 1843 and the Woburn Experimental Station founded by the Duke of Bedford in 1876, were chiefly concerned with problems regarding the production of cereals like wheat, barley etc. and meat.

In the following table it will be shown that the production of these materials is much less economical than the production of milk, potatoes, sweet potatoes, etc. which leave healthy alkaline residues in the body whilst cereals and meat make the system acidic, and this is harmful.

By the application of a mixture of inorganic and organic manures and sound soil management the prduction of potatoes and sweet potatoes is very high in Europe. The amount of energy available per acre with these tubers is much greater than with cereals like wheat, barley etc.

TABLE XXIII

Average yield increase produced by one ton of fertilizer.

Crop.	Yield increase.	Crop	Yield increase.
Corn	125 bushels	Beef	1000 lbs.
Cotton	2 bales	Milk	8000 lbs.
Wheat	85 bushels	Apples	700 busheles
Oats	140 ,,	Snap beans	130
Tobacco	1,370 lbs.	Tomatoes	215
Potatoes	185 bushels	Peanuts	200
Sweet potatoes	285 ,,	Soy beans	50

CALORIC AND PROTEIN INTAKE OF DIFFERENT COUNTRIES

The most important factors in human diets are the caloric intake and the amount of protein consumed. Every day a human being carrying on ordinary work requires approximately 2700 calories for his existence and work. The golden rule is that you can easily determine your caloric need by multiplying the body weight by 35. If a large amount of muscular work has to be performed, the caloric need increases.

Experts have also decided that a human being requires a definite amount of proteins for maintaining good health. Here again, the golden rule is that the protein requirement of your body in grams per day is the same as your body weight in seers (kilograms). In other words, a person whose weight is 70 seers (kilograms) requires 70 grams of protein, *i.e.* a little more than one 'chhatak' of protein per day. In recent years quantitative work on the protein requirements of human beings and animals has been carried on with great intensity all over the world. These researches have definitely established that out of 70 grams needed by a man per day, one half, *i.e.* 35 grams, must be of animal origin which is obtainable from milk, fish, egg, meat etc. The other half of the protein can be derived from vegetable sources, *i.e.* pulses, wheat, rice, potatoes and other vegetables.

It is clear therefore that the standard caloric requirement of a human being is 2700 calories per day and the protein requirment is 70 grams of which 35 grams must be from animal source. Hence, for 2500 million inhabitants of the world 35 million tons of animal proteins and the same amount of vegetable proteins are needed. The world production of pulses is 17.9 million tons containing 2.4 million tons of total nitrogen. It is of interest to record here that the world production of wheat is 142 million tons per annum containing 2.4 million tons of total nitrogen. The 70 million tons of proteins needed per year for the whole world contain 11 million tons of total nitrogen. There are 125 million acres under legumes which can add 5 million tons of total nitrogen to the soils of the world per year.

Let us see what the experts of the F.A.O. have concluded from the study of food habits and food standards of different countries.

TABLE XXIV

"Energy and protein content of estimated national average food supplies available for human consumption during the year 1949-50.

Country.	Calories.	Total protein.	Animal protein.	Country.	Calories.	Total protein.	Animal protein.
North America.				*Europe.*			
				Austria	2610	76.0	30.0
Canada	3130	93	56.1	Belgium&Luxemburg	2895	85.0	41.0
U.S.A.	3170	91	60.4	Denmark	3160	102.0	59.0
South America.				Finland	3100	96.0	43.0
Argentina	3169	102.6	66.7	France	2685	97.0	41.0
Brazil	2444	65.2	24.9	Germany (F.R.)	2660	79.0	33.0
Chile	2340	70.4	22.9	Germany (S.Z.)	2460	72.0	19.0
Colombia	2328	56.7	25.6	Greece	2490	77.0	19.0
Peru	2276	63.8	13.4	Ireland	3340	97.0	50.0
Urugnay	2623	94.4	62.1	Italy	2370	75.0	20.0
Venezuela	2210	63.2	27.8	Netherlands	2970	87.0	39.0
Far East.				Norway	3135	102.0	56.0
Ceylon	2010	44.0	11.0	Sweden	3200	94.0	60.0
China	2020	62.0	5.0	Switzerland	3200	94.0	60.0
India	1700	43.0	6.0	United kingdom	3080	91.0	46.0
Indo-China	1560	37.0	5.0	*Oceania.*			
Indonesia	1880	42.0	5.0	Australia	3210	98.0	66.7
Japan	2100	53.0	8.0	New Zealand	3400	101.0	65.8
Philippines	1970	45.0	10.0	*Central America*			
Near East.				*and Carribean.*			
Egypt	2360	69.6	9.5	Cuba	2822	67.6	25.3
Turkey	2340	73.5	17.0	Mexico	2052	55.6	16.1

From the foregoing table it is quite clear that the present food standard of our country is definitely worse than what it was 25 years ago. Our Government should look into this matter as soon as possible with all earnestness. One of the important reasons of this acute shortage is that our people depend mainly on cereals and on vegetables as is done in modern times in many countries of Europe.

Out of the world population of 2500 million, India including Pakistan and China possesses almost half of humanity. Unfortunately, food production in these countries specially has lagged behind population increase, and hence, the outcome is a disastrous food shortage. The world production of cereals on which the poor people of the East have to depend almost entirely for their daily bread is 600 million tons per year which is adequate even today for whole world population; but this country and Pakistan, possessing 1/5th of the world population, produce only 1/10th of the cereals, *i.e.* only 60 million tons per annum.

In Germany and France 400 lbs. of potatoes are taken by an adult per year, in Denmark 300 lbs., and in England 200 lbs. Potatoes not only supply carbohydrates which produce energy to carry on work but are rich in minerals like potash, soda, lime, magnesia, phosphate—materials which are builders of teeth, bones and tissues. Before the intrduction of pototoes in Europe, the Europeans consumed plenty of bread, meat, fish, egg and wines which are acid-producing materials harmful to the body. Today potatoes and milk are largely consumed along with smaller amounts of other food materials and the diet is balanced and healthy, and thus the general health of the people is excellent although they grumble that they do not get enough meat.

It is well known that the plants which produce pulses are endowed with the wonderful property of fixing nitrogen of the air with the help of the bacteria, Rhizobia. The combination of the plant and the bacteria is an efficient method of enriching the soil, and the efficiency of this type of nitrogen fixation is 25% or more.

It has been frequently reported that in these leguminous plants like pulses, clover, alfalfa, 'sanai' (sunhemp), vetch, lucerne etc. 100 milligrams of nitrogen are fixed per gram of carbon oxidised. Hence, these leguminous plants are being largely utilised all over the civilized world either in crop-rotation or in enriching the soil from the nitrogen point of view. These legumes are rich in nitrogen and serve as an excellent fodder for cows and thus improve milk production. It has been estimated that in U.S.A. alone, 38 million acres of land are under legumes which add two million tons of nitrogen to the American soil. This practice of putting land under legumes is rapidly increasing all over the world and should be taken up by this country on a large scale immediately. Where legumes are not available or are difficult to grow, all kinds of organic matter, plant residues, straw, leaves, peat, lignite, powdered coal, which is being wasted today, should be added to the land which should be ploughed so that the organic matter be well mixed, and after 3 or 4 months crops such as paddy, potatoes, sweet potatoes, tomatoes can be grown with great profit. Today lignite is being mined from Palana in Madras State, but so far this material has not been used as a fuel. Our experiments show clearly that lignite and Assam coal when mixed with soil not only fix atmospheric nitrogen, thus enriching the soil, but can also reclaim alkali and 'usar' land.

In the foregoing pages it has been stated that plants not only require water, calcium, potash, phosphate and nitrogen, but a large number of elements in small quantities are also needed for their growth. The artificial fertilizers like ammonium sulphate, superphosphate, and potassium chloride or sulphate, which are largely used in European and American agriculture, are not only much more costly than the waste products of commerce and agriculture like molasses, pressmud, sawdust, leaves, straw, powdered coal etc., but these organic materials can not only fix atmospheric nitrogen and protect the soil nitrogen from loss, but also add minerals like potash, phosphate, iron, manganese, boron, molybdenum, vanadium and other rare elements needed for the healthy growth of vegetables and animals.

Today the synthetic nitrogenous compounds are costlier than superphosphate or potash because the latter are usually manufactured from rocks or products occurring

in nature. The farmers using only organic substances as manures report that artificial fertilizers are too costly for crop production. Moreover, research work carried out in the Allahabad University has elucidated the science of manuring by organic matter which is coming into general use all over the world today.

Organic substances like straw, leaves, etc. are rich in potash which is much more profitable to crops like potatoes, lettuce, celery sweet potatoes, tomatoes, beetroot etc. than in wheat, oats, barley, etc. Hence, man has to decrease the amount of wheat consumed and depend more on excellent materials like potatoes, sweet potatoes, tomatoes, celery for healthy existence. Moreover, meat production is approximately six times costlier than milk production. Thus, man has to depend more on milk and its products than on meat.

The world food situation is bound to be more and more difficult and acute as its production is not keeping pace with increase of population. Hence, men all over the world have to take more of sweet potatoes, tomatoes, carrots for their caloric and vitamin need and milk for the good quality protein, calcium etc. It is no wonder, therefore, that the European or the American who depended chiefly on meat, fish, egg and bread has now to depend more on tubers and milk, thus becoming a vegetarian. This important change in food habit may perhaps be for the good of humanity and decrease the possibility of the occurrence of war between man and man. It is interesting to note that the eminent Swedish physiologist, Hindhede, lived a healthy life for several years with a diet consisting of 3 kg. of potatoes per day and small amounts of milk and margarine.

TABLE XXV

Nutrient content of some field & horticultural crops.

Crop.	Yield.	Part of crop.	Nitrogen. (lbs.)	Phosphate. (lbs.)	Potash. (lbs.)	Total (lbs.)
Cotton	500 lbs.	lint				
	1000	seed	38	18	14	70
	1500	burrs, leaves, stalks	27	7	36	70
		Total :	65	25	50	140
Tobacco	1500	leaves	55	10	80	145
		stalks	25	10	35	70
		Total :	80	20	115	215
Corn	60 bushels	grain	57	23	15	95
	2 tons	stover	38	12	55	105
		Total :	95	35	70	200
Wheat	30 bu.	grain	35	16	9	60
	1.25 tons	straw	15	4	21	40
		Total :	50	20	30	100
Oats	50 bu.	grain	35	15	10	60
	1.25 tons	straw	15	5	35	55
		Total :	50	20	45	115
Barley	40 bu.	grain	35	15	10	60
	1 ton	straw	15	5	30	50
		Total :	50	20	40	110

TABLE XXV (contd.)

Crop.	Yield.	Part of crop.	Nitrogen. (lbs.)	Phosphate. (lbs.)	Potash. (lbs.)	Total (lbs.)
Potatoes	300 bu	tubers	65	25	115	205
		tops	60	10	55	125
		Total :	125	35	170	330
Sweet potatoes	300 bu.	roots	45	15	75	135
		vines	30	5	40	75
		Total :	75	20	115	210
Tamatoes	10 tons	fruit	60	20	80	160
		vines	40	15	95	150
		Total :	100	35	175	310
Sugar beets	15 tons	roots	55	22	53	130
		tops	60	23	92	175
		Total :	115	45	145	305
Cabbage	15 tons	all	100	25	100	225
Celery	350 crates	all	80	65	235	380
Spinach	9 tons or 1000 bu	all	90	30	45	165
Apples	400 bu.	fruit	20	7	30	57
		leaves, wood	10	3	5	18
		Total :	30	10	35	75
Peaches	50 bu.	fruit	30	15	55	100
		leaves, wood	55	10	45	110
		Total :	85	25	100	210
Grapes	4 tons	fruit	10	6	20	36
		leaves, canes	15	4	15	34
		Total :	25	10	35	70
Oranges	600 boxes	fruit	65	23	105	193
		leaves, wood	25	7	25	57
		Total :	90	30	130	250
Soy beans	25 bu	grain	110	35	40	185
	1.25 tons	straw	15	5	20	40
		Total :	125	40	60	225
Peanuts	1 ton	nuts	60	10	10	80
	3 tons	vines	25	5	40	70
		Total	85	15	50	150
Pea beans	30 bu	grain	73	23	24	120
		straw	22	7	31	60
		Total :	95	30	55	180
Alfalfa	3 tons	all	140	35	135	310
Sweet clover	5	,,	185	45	165	395
Red clover	3	,,	80	20	70	170
Lespedeza	3	,,	130	30	70	230
Cow peas	2	,,	125	25	90	240
Timothy	1.5	,,	40	15	45	100

Very careful experiments have been carried out in Denmark about the growth of various crops by the use of farmyard manure containing definite amounts of nitrogen, phosphorus and potash in comparison with artificial fertilizer containing equivalent amounts of sodium nitrate, superphosphate and potassium chloride. The results are most interesting and the average for 50 years shows that potatoes and swedes grow appreciably better, and greater yields are obtained with farmyard manure than with artificials. The yield of oats is slightly smaller with farmyard manure than with artificials, but the yield of wheat, rye and barley is much less in farmrard manure than with artificials. It is clear therefore that yields of crops differ a good deal according to the nature of crops. The reason of this behaviour appears to be as follows:-

The amount of nitrogen needed by wheat is much greater than its need for potash or phosphate. On the other hand, potatoes and other tubers and roots require more potash than nitrogen. It is well known that the potash content of farmyard manure, or even straw, is greater than the phosphate or the nitrogen content. Moreover, it is generally believed that the availability of potash is certainly as high from organic manures as from inorganics, because potash is believed to exist in soluble form in organic manures. The availability of phosphate is more or less the same from organic and inorganic sources, but the quick availability of nitrogen is much less pronounced in organic than in inorganic manures. The Danish experiments seem to show that in a 4 or 5 year rotation 32 to 40% of the nitrogen of the organic manure is withdrawn and is available to crops whilst 60 to 70% of nitrogen in inorganics is taken up by the crop. Hence, it can be concluded that crops whose need of nitrogen is more pronounced than that of potash or phosphate should respond better with artificials than with organic manures. This conclusion is supported by the nutrient content of some field and horticultural crops as recorded in the foregoing table.

In case of legumes there is a marked nitrogen fixation in nodules, and it is generally believed that approximately $\frac{2}{3}$ to $\frac{1}{2}$ of the nitrogen content of these plants is derived from atmospheric nitrogen by the fixation process; hence, the amount of nitrogen extracted from the soil is much less than the amount of potash taken up from the soil. Hence, legumes grow well with farmyard manure. As a matter of fact the Danish experiments show that clover is produced in greater quantities with farmyard manure than with artificials.

It is interesting to record here that from ancient times two viewpoints regarding plant nutrition have been advanced. Barnard, Palissy Bacon, Glauber, Boyle, and specially Liebig, were supporters of the theory, first emphasised by Paracelsus, that salts were the true nutrients of plants. On the other hand, there were several distinguished men, notably, Home, Wallerius, Thaer, de Saussure, Davy, de Candolle, Berzelius, Mulder and others, who were advocates of the Aristotlean doctrine of plant nutrition by soil organic matter.

The Western scientists, being industrially minded, have manufactured ammonium salts, urea, calcium nitrate, superphosphates and potash salts and are applying them as manures. In the East, however, artificial fertilizers have not been in vogue. However,

the Europeans have now realised that artificial manures are unable to cope with the world food production, and they have to acknowledge the failure of chemical technology in this respect.

Hence, eminent soil scientists of Europe and America have taken great interest in our researches on the value of organic matter in nitrogen fixation and nitrogen loss as recorded in this paper. Sir John Russell commented as follows in "Nature" (April 11, 1936, page 619):—

"Professor Dhar leads the school of thought which believes that nitrification in soils and nitrogen fixation from the atmosphere are, especially in the tropics, photochemical, at least as much as bacterial action. Prof. Dhar has produced strong evidence in support of his theories".

"The practical facts of Prof. Dhar's researches are that Indian soils are generally deficient in nitrogen, that more than half a million tons of molasses from the sugar industry are annually wasted in India, and that the application of molasses to the soil can double, and may treble, the soil nitrogen content with a consequent large increase in crop yield".

"Prof. Dhar has produced indisputable evidence of increase in available soil nitrogen and crop yields following the application of molasses. Under temperate conditions the converse result would be expected as it is well known that the addition of carbohydrate-rich material to the soil tends to reduce the amount of nitrogen available to plants, the nitrogen becoming fixed as microbial protoplasm or as humus. As essential difference, however, between temperate and tropical soil requirements, is that whereas in temperate regions the limiting factor to crop growth is often the slowness, in the tropics it is the rapidity with which the soil nitrogen is made available to plants, soluble nitrates being formed and leached from the soil before they can be absorbed by the crop. The general effect of molasses on the soil should be the same everywhere, but only in tropics will its 'braking' effect on the mobilisation of soil nitrogen be a positive advantage to the cultivator, and only in the tropics will its stimulation of atmospheric nitrogen fixation, whether bacterial or photochemical, be appreciable, since temperate regions lack the heat necessary for bacterial and the light for photochemical stimulation".

"From these reasons the potentialities of carbohydrate manuring—of which molasses manuring is an example—have, perhaps, been overlooked by agriculturists. If Prof. Dhar can substantiate his claims, he may effect a revolution in agriculture in India where supply of the ordinary organic manures is far below the demand. Prof. Dhar suggests that a most valuable use can be made of molasses in reclaiming alkaline land. The acids produced in the decomposition of molasses neutralise the alkalies, and at the same time and contrary to experience when land is reclaimed with gypsum or sulphur, soil nitrogen is increased".

"There are 4 million acres of infertile alkaline land in India and irrigation practices are increasing the area. The economic reclamation of these lands is one of country's greatest agricultural problems to the solution of which Prof. Dhar's work is pointing the way".

Similarly Mr. J. I. Rodale of Pennsylvania, in "The Farmer" (1949, Winter Issue, page 10), has stated as below :

"To say that this is an exciting situation is to put it mildly. But if Dr. Dhar is right, and he seems to have done the experimental work to prove it, we seem to be on the threshold of something spectacular. The first phase of work was accomplished by Sir Albert Howard in India. Here is another phase which was done in that same country. Since Dr. Dhar is the Head of an important Department in a recognised University, I feel certain that there must be some substance to the letter just quoted. The new method is a combination of Sir Albert Howard and Dr. Dhar".

"What is the significance of this letter ? In the case of making compost by the aeration method, some of the nitrogen is lost. At any rate the total nitrogen content of compost is not very high about one to two per cent. By placing our organic matter in a thin layer in the field and discing it in the upper 4 inches, there is a much slower oxidation than in the composting process, and in the field, says Dr. Dhar, more nitrogen is fixed. A careful perusal of his papers gives confidence in his methods and findings".

The Editor of "Farmer", Mr. Newman Turner, has recorded as follows:—

"..... But Dr. Dhar's findings are thoroughly sound. We have practised them for nearly 7 years at Goosegreen Farm with great success".

Dr. Charlotte M. Hoak of 1125 Stratford Avenue, South Pasadena, California, U.S.A. has put the position as under:

"..... It is with profound interest that I have read and digested as far as possible, with my limited experience, the helpful chemical research in agricultural fields done by your leader, Dr. N. R. Dhar, Head of the Chemistry Department of the University of Allahabad. The various reprints of the Proceedings of the National Institute of Sciences, India, and his invaluable book on Biochemistry should be in the hands of every progressive agriculturist".

"Of special interest to us in the semi-arid Southwest is the 'Reclamation of *Usar* (alkaline) Land by treatment of Molassess and Pressmud', Of great interest througout the country is the new light thrown on the direct nitrification of the soil".

Hence, a good deal of interest is being shown in the value of organic matter in land improvement. As many farms have no animals now due to the introduction of tractors, straw is abundant in such farms; straw mixed with calcium phosphate or basic slag is likely to prove an excellent manure, specially for the production of tubers, vegetables, pulses and other legumes.

In 1911, my Guru introduced me to ammonium nitrite when its vapour density was determined by us. Since that time this substance has been studied by me and my pupils. In 1920 its decomposition was found to be accelerated by light absorption. We have been able to show that the formation and decomposition of this substance play a very important role in soil fertility and cultivation and in the nitric acid industry.

JAI HIND

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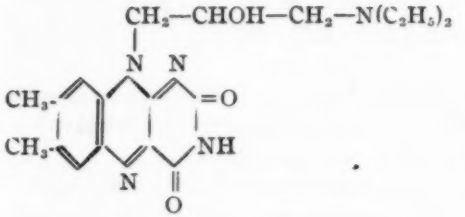
ANTIMALARIALS IN THE QUINAZOLINE SERIES. PART II

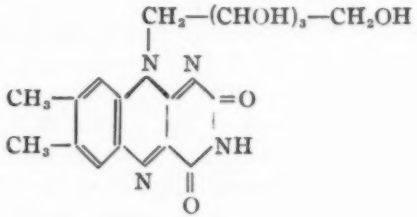
By (Miss) Shakti Rani, O.P. Vig, I. S. Gupta and K. S. Narang

Some quinazolone derivatives have been prepared with view to testing their antimalarial activity.

Atebrine shows reversal of the metabolic activity of riboflavine (Silverman and Evans, *J. Biol. Chem.*, 1943, **150**, 265). From this it would appear that antimalarials may in part act by virtue of their antimetabolite action. It has been established (Powers, "Advancing Fronts in Chemistry", Vol. II, pp. 68-69) that the compound (I) and other basically substituted alloxazine derivatives do not show any tendency to reverse the metabolite effect of riboflavine (II).

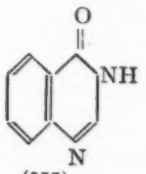


(I)

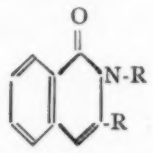


(II)

From this we are inclined to conclude that for any metabolite-antimetabolite action, the antimetabolite compounds need have certain common and certain different structural features as in sulphanilamide and *p*-aminobenzoic acid, and the failure of alloxazine derivatives to have any antimetabolite action to riboflavine may be due to their too close a structural relationship to riboflavine. We therefore consider it worthwhile to study the effect of structural types which may have less common features with riboflavine than the alloxazine derivatives. Quinazolone-4 (III) is one such structural type.



(III)



(VI)

[R and R′, various substituents]

It is significant to note that febrifugine and *iso*febrifugine, the alkaloids from the well known *Dichroa Febrifuga*, Lour is a quinazolone-4 derivative with a basic side chain at position 3 (*J. Amer. Chem. Soc.*, 1949, **71**, 1048). The authors therefore have synthesised new quinazolone-4 derivatives with side chains at positions 2 and 3 of general type (IV) in order to study their antimalarial activity.

The quinazolone derivatives have, except where described otherwise in the experimental part, been prepared by heating together the appropriate acylanthranilic acid, the amines and phosphorus trichloride in toluene.

EXPERIMENTAL

2-Methyl-3-p-methoxyphenylquinazolone-4.—N-Acetylanthranilic acid (1 mol.,7 g.) and *p*-anisidine (1 mol., 4.8 g.) were taken in 150 c. c. dry toluene. A solution of 3 c. c. of phosphorus trichloride in 12 c. c. dry toluene was added to the above during 15 minutes with stirring when a thick white precipitate was formed. The mixture was heated with stirring at 130°-135° for 2 hours. After cooling the toluene was filtered off and the residue was taken in 250 c.c. of water containing 10 c.c. of strong hydrochloric acid. After boiling for a few minutes the product dissolved in acidulated water. After filtering and cooling, the clear solution was basified with Na_2CO_3 when the base separated out as a white sticky mass which solidified on trituration and cooling in ice. It was crystallised from 80% alcohol (charcoal) and recrystallised from petroleum ether (b.p. 80°-100°) in colorless rhombic crystals, m.p. 168°, yield 5 g. (Found : C, 71.73 ; H, 5.01. $C_{16}H_{14}O_2N_2$ requires C, 72.17; H, 5.26 per cent).

The base was converted into the hydrochloride by dissolving in hot absolute alcohol and acidifying with a few drops of HCl (conc.). The hydrochloride on crystallisation from absolute alcohol was obtained as a white powder, m.p. 260° (decomp.).

All the quinazolone derivatives (the *p*-hydroxyquinazolones excepted), listed below, have been prepared by the same method using acylanthranilic acid (7 g.), 1 mol. of amine, 150 c.c. of toluene and 3 c.c. of phosphorus trichloride in each case. All hydrochlorides were crystallised from absolute alcohol.

The 3-*p*-hydroxyphenylquinazolones were prepared by refluxing 3 g. of the corresponding methoxy derivatives with a mixture of 30 c.c. of 48% hydrobromic acid and 75 c.c. of acetic anhydride till a test portion was found to be completely soluble in sodium hydroxide solution. The solution was then concentrated in vacuum and the residue dissolved in sodium hydroxide and filtered to remove any insoluble impurity. The filtrate was made just acidic with acetic acid. The colorless solid so obtained was purified by solvents mentioned in the table.

TABLE 1

Q stands for -quinazolone-4.

Name.	Solvents for crystallisation.	M.p. of the base.	M.p. of Hydrochloride.	Yield of the base.	Base Found.	Base Calc.
3-(*p*-Methoxyphenyl)-Q	1. EtOH 2. Pet. ether	186°		4.0 g.	N, 11.4%	N, 11.11%
*3-(*o*-Methoxyphenyl)-Q	...	...	222° (d)	3.5	N, 9.85	N, 9.70
3-(*p*-Ethoxyphenyl)-Q	1. EtOH 2. Pet ether	158°	234° (d)	4.5	N, 10.70	N, 10.52
*3-(*o*-Ethoxyphenyl)-Q	...	...	220° (d)	3.8	N, 9.05	N, 9.25
3-(*p*-Chlorophenyl)-Q	1. EtOH 2 Pet. ether	176°	236° (d)	5.0	N, 10.6	N, 10.92

TABLE I (*contd.*)

Name.	Solvents.	M.P. of the base	M.P. of hydrochlorides.	Yield of the base	Base. Found.	Base. Calc.
*3-Benzyl-Q	...	...	230°	2.5 g.	N, 10.80%	N, 10.60%
3-(*p*-Hydroxyphenyl)-Q	1. EtOH 2. Benzene	212°	244° (d)	2.2	N, 11.40	N, 11 76
2-Methyl-3-(*p*-chloro-phenyl)-Q	1. EtOH 2. Pet. ether	155°	270° (d)	5.2	C, 66.51 H, 3.98 Cl, 12.85	C, 66.45 H, 4.06 Cl, 13 12
2-Methyl-3-(*o*-methoxy-phenyl)-Q	1. EtOH 2. Pet. ether	132°	282° (d)	4.5	C, 71 80 H, 5.35	C. 72.17 H, 5.26
*2-Methyl-3-(*o*-ethoxy-phenyl)-Q	...	...	197° (d)	4.6	N, 8.5	N, 8.84
2-Methyl-3-(*p*-ethoxy-phenyl)-Q	1. EtOH 2. Pet. ether	156°	270° (d)	4.7	N, 10.07	N, 10.0
*2-Methyl-3-benzyl-Q	...	...	230° (d)	3.0	N, 9.61	N, 9.77
2-Methyl-3-(*p*-hydroxy-phenyl)-Q	1. EtOH 2. Benzene	278°	Did not melt upto 350°.	2.5	N, 10.90	N, 11.11
2-Ethyl-3-(*p*-chloro-phenyl)-Q	1. EtOH 2. Pet. ether.	185°	230°	6.2	C, 67.56 H, 4.51	C, 67.48 H, 4.56
2-Ethyl-3-(*o*-ethoxy-phenyl)-Q	1. EtOH 2. Pet. ether.	117°	220°	6.0	N, 9.7	N, 9.52
2-Ethyl-3-(*p*-ethoxy-phenyl)-Q	1. EtOH 2. Pet. ether	165°	206°	6.1	C, 73 50 H, 6.00	C, 73.47 H, 6.12
2-Ethyl-3-(*o*-methoxy-phenyl)-Q	1. EtOH 2. Pet. ether	143°	211° (d)	6.0	N, 9.90	N, 10 00
2-Ethyl-3-(*p*-methoxy-phenyl)-Q	1. EtOH 2. Pet. ether	145°	252° (d)	5.9	N, 10.20	N, 10.00
2-Ethyl-3-benzyl-Q	1. EtOH 2. Pet ether	110°	196° (d)	5.0	N, 10.30	N, 10.60
2-Ethyl-3-(*p*-hydroxy-phenyl)-Q	1. EtOH 2. Benzene	261°		2.5	N, 10.40	N, 10 52
2-*n*-Propyl-3-(*p*-chloro-phenyl)-Q	1. 75 % EtOH 2. Pet. ether	132°	220° (d)	7.5	C, 68.06 H, 5.19 N, 9.45	C, 68.34 H, 5.25 N. 9.38
2-*n*-Propyl-3-(*o*-methoxy-phenyl)-Q	1. 75% EtOH 2. Pet. ether	101°	216° (d)	6.5	N, 9 35	N, 9.52
2-*n*-Propyl-3-(*p*-ethoxy-phenyl)-Q	1. 75% EtOH 2. Pet. ether	129°	206°	6.5	N, 8.82	N, 9.09
2-*n*-Propyl-3-(*o*-ethoxy-phenyl)-Q	1. 75% EtOH 2. Pet ether	102°	203°	6.95	N, 8.84	N, 9.09
2-*n*-Propyl-3-(*p*-methoxy-phenyl)-Q	1. 80% EtOH 2. Pet ether	182°	308°	6.50	N, 9.84	N, 9.52
2-*n*-Propyl-3-benzyl-Q	1. 75% EtOH 2. Pet ether	92°	184°	4.0	N, 9.85	N, 10 07
2-*n*-Propyl-3-(*p*-hydroxy-phenyl)-Q	1. 80% EtOH 2. Pet. ether	221°		2.06	N, 10.30	N, 10.0

* In these cases the bases separated in liquid form and were extracted with ether and converted into hydrochlorides by passing dry hydrochloric acid gas; the analysis and yields are those of the hydrochlorides.

2-Methyl-3-(piperidino-n-propyl)-quinazolone-4 Dihydrobromide.—2-Methyl-3 : 1 : 4-benzoxazone (3 g.) (Zentmyer and Wagner, *J. Org. Chem.*, 1949, **14**, 967) and piperidino-*n*-propylamine (3 g.) were heated on a steam-bath for 3 hours and subsequently at 180° under reduced pressure. The dark brown product was extracted with ether. The hydrobromic acid gas was passed through the ethereal extract. The dihydrobromide was obtained as a pale brown powder on crystallisation from absolute alcohol, m.p. 248°, yield 3.0 g. (Found: N, 9.07. $C_{17}H_{25}ON_3Br_2$ requires N, 9.39 per cent).

The authors wish to express their thanks to Dr. S. M. Mukherji for his keen interest in the investigations.

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ROLE OF ANTIOXIDANTS DURING STABILISATION OF VITAMIN-A IN SHARK LIVER OIL

By G. G. Kamath and N. G. Magar

Antioxidants, N. D. G. A. and propyl gallate, effectively stabilise vitamin-A from shark liver oil as observed by aeration and storage. 2:6-Di-*tert.*-butyl-*p*-cresol is slightly less effective. B. H. A. is ineffective.

The Waghbeer liver oil, containing a small amount of vitamin-A is more stable than Khada Mushi or Pisori liver oils containing higher amounts of vitamin. Antioxidants are more effective at lower temperatures of storage, if the oil contains negligible peroxide. In ultraviolet light, the antioxidants lose the protective property and vitamin-A is destroyed rapidly.

The problem of the stabilisation of vitamin-A in fish liver oils by different antioxidants has been investigated by many workers. Ramaswamy and Banerjee (*Ann. Biochem. Expt. Med.*, 1948, **8**, 115) have observed that nearly 83% of the total vitamin-A in shark liver oil is destroyed on storage for 35 days. *iso*Butyl gallate (0.02 %) with tartaric acid has been found to be the best antioxidant for shark liver oils by Bose and Banerjee (*Ind. J. Med. Res.*, 1945, **33**, 203) and Patel and Sreenivasan (*J. Sci. Ind. Res.*, 1950, **9B**, 99).

Dassow and Stanby (*J. Amer. Oil Chem. Soc.*, 1949, **26**, 475) found that 0.1% N. D. G. A. stabilised vitamin-A in Halibut liver oil at lower temperatures but even 0.1–0.5% of N. D. G. A. was ineffective at 97.7°. Kern, Antoshkiw and Maiess (*Ind. Eng. Chem.*, 1949, **41**, 2849) noted that a mixture of 4% lecithin and 2% tocopherol was an effective antioxidant for fish liver oils. Alkyl gallates have also been found to afford protection to shark liver oils (cf. Bittenbender, *Comm. Fish. Rev.*, 1950, **12**, No. 2). This investigation deals with the stabilisation of vitamin-A in shark liver oils by some of the recently introduced antioxidants.

Experimental

The oils for investigation were extracted from livers of sharks caught off the Bombay coast. The identified sharks were cut open and the livers washed free of adhering blood. The livers were then minced and the pulp cooked with one third of its weight of water at 50° on a water-bath in a stainless steel vessel. The oily layer was then separated, kept overnight with anhydrous Na_2SO_4, filtered and used for investigation. The free fatty acids were negligible in these samples.

The antioxidants used were N. D. G. A., propyl gallate, 2: 6-di-*tert.*-butyl-*p*-cresol and butylated hydroxyanisole (soild and liquid). N. D. G. A. and propyl gallate were dissolved in a small quantity of ethyl alcohol and then incorporated in the oil.

Estimation Vitamin-A.—A weighed amount of the oil was dissolved in redistilled chloroform. To 1 ml. of this solution, 4 ml. of the activated G. D. H. were added, shaken, and the intensity of the pink colour developed was measured after 3 minutes in a Klett Summerson photoelectric colorimeter using a green filter (500-570 mμ).

The readings were converted into I. U./g. of vitamin-A from a standard graph plotted with the same activated G. D. H. and standard vitamin-A, supplied by the Distillation Product, U. S. A. (200,000 I. U./g.).

Vitamin-A was also estimated in the later part of the work (vide Tables VI-VIII) with the Beckman absorption spectrophotometer (model DU) by multiplying $E^{1\%}_{1cm.}$ at 328 mμ with 1600, using ethyl alcohol as the solvent.

Peroxide Number.—Wheeler's iodometric method for the estimation of peroxide number was found to be unsatisfactory in fish liver oils, and hence, the method of Chapman and Mackay (*J. Amer. Oil Chem. Soc.*, 1949, **26**, 360) was adopted. A known amount of the oil was dissolved in a 70:30 v/v mixture of chloroform and alcohol, so that 1 ml. contributed about 10 peroxide numbers. To 1 ml. of this solution, 8.9 ml. of the solvent mixture, one drop of NH_4CNS solution (30 g./100 ml. H_2O) and one drop of ferrous chloride solution (0.4%) were added. The contents were shaken, kept at 50° for 2 minutes and then cooled to room temperature. A stable violet colour was developed the intensity of which was noted with the colorimeter, using a green filter. The reading obtained was computed with standard ferric ion solution and the peroxide number calculated from the formula:—

$$\text{Peroxide No. m.e. per kg. of oil} = ab/55.84\,c,$$

where a = net μg. of Fe, obtained from standard graph, b = vol. of original dilution in ml., and c = wt. of oil taken, 55.84 being the equivalent weight of Fe.

The iodine values of the oils were found by the standard A.O.A.C. Wij's method.

Stability Tests.—Test tubes containing oil samples were held in a water-bath maintained at 55°. Air, purified by passing through solutions of $K_2Cr_2O_7$ and KOH was then bubbled at a regulated rate through the oil samples. The samples were tested periodically for vitamin-A and peroxide numbers.

Storage Experiments.—The oil samples in pyrex test tubes, plugged with cotton and kept in closed wooden boxes so as to protect them from light, were stored at room temperature, as well as at 38°.

TABLE I

Percentage destruction of vitamin-A in Khada Mushi liver oil on aeration at 55° ± 5°.

	Aeration period (hours).					
	29.	50.	72.	84.	92.	100.
Control sample	11.9	17.5	30.8	73.7	100	100
NDGA (0.02%)	2.2	16.5	25.6	...	38.0	38.0
Propyl gallate (0.02%)	2.3	17.0	26.0	...	41.4	41.4
D-b-*p*-cresol „	6.6	18.1	33.3	45.9	51.6	68.5
B.H.A (liquid) „	6.5	21.5	45.7	66.1	81.3	100
B.H.A (solid) „	7.3	14.5	43.5	68.6	80.3	100

TABLE II

Peroxide No. for Khada Mushi liver oil on aeration at 55° ± 5°.

		Aeration period (hours).					
		29.	50.	72.	84.	92.	130
Control sample		15	24	45	121	323	550
NDGA	(0.02%)	11	16	25	36	41	43
Propyl gallate	,,	10	13	23	38	48	53
D-b-*p*-cresol	,,	14	20	40	54	60	61
B.H.A. (liquid)	,,	17	27	52	85	121	175
B.H.A. (solid)	,,	16	26	51	82	112	152

DISCUSSION

Stability of Shark Liver Oil on Aeration

Tables I and II show the vitamin-A destruction and development of peroxides with Khada Mushi (*Carcharinus melanopterus*) liver oil, which had an initial vitamin-A content of 29,500 I. U./g. and peroxide number of 10. The order of efficiency of the antioxidants can be seen by comparing the vitamin-A destruction with the peroxides developed. Further, B.H.A. (liquid and solid) gave about 6.5 and 7.3% destruction of the original vitamin-A after 29 hours' aeration as compared to 11.9% destruction in the untreated sample. With further aeration, however, only 38% of the total vitamin-A was destroyed by aeration for 92 hours. The peroxide number reached was only 43 as compared to 550, of the untreated sample. Propyl gallate follows N.D.G.A. very closely in the retention of vitamin-A and formation of peroxides. D-b-*p*-cresol also shows antioxidant properties and ranks after propyl gallate. These observations compare very favourably with those of Moore and Bickford (*J. Amer. Oil Chem. Soc.*, 1949, **26**, 449) who have noted that the order of the effectiveness of antioxidants in lard is (1) propyl gallate, (2) N.D.G.A., (3) di-*tert.*-butyl-*p*-cresol and (4) B.H.A. at 0.05% and 0.1% concentration ; the last was also found to be ineffective in frying with lard and not carried over, by Sair and Hall (*Food Technol.*, 1951, **5**, 69).

Comparison of Waghbeer and Pisori Liver Oils

Table III shows that though Waghbeer (*Galeocerdo tigrinus*) liver oil contains a small amount of vitamin-A, it is far more stable than the Pisori (*Carcharinus limbatus*) liver oil containing high amount of the vitamin. The average degree of unsaturation indicated by the iodine number is only 68.5 for Waghbeer liver oil, whereas that for Pisori is 94.5. Thus, Pisori liver oil will contain more highly unsaturated fatty acids than Waghbeer liver oil. Hilditch (*Chem. Ind.*, 1944, **8**, 67) has already observed that fish liver oils which contain highly unsaturated fatty acids become rancid easily if exposed to air, and that this oxidation may lead to vitamin-A destruction.

TABLE III

Percentage destruction of vitamin-A in Pisori and Waghbeer samples on aeration at 97° ± 2°.

	Initial vitamin-A (I.U./g.).	Aeration period (hours).			
		3.5.	10.5.	18.	24.
Pisori (female)	35,650	7.8	21.1	74.8	92.8
Pisori (male)	50,750	3.8	61.0	93.2	...
Waghbeer (female)	2,560	7.8	15.2	26.6	72 9
Waghbeer (male)	1,690	...	0.4	0.8	21.0

The stability of Waghbeer liver oil towards vitamin-A may also help in improving the retention of vitamin-A in liver oils with higher unsaturation. A mixed sample of Khada Mushi and Waghbeer liver oils was therefore aerated with d-b-*p*-cresol and propyl gallate. The initial vitamin-A content of the oil was 12,000 I. U./g. and peroxide number, 11. From Table IV it can be concluded that this mixed sample of oil is more stable than the Khada Mushi sample (vide Tables I and II) as only 40% destruction of the original vitamin-A is noticed after aeration for 100 hours. Both d-b-*p*-cresol and propyl gallate, however, failed to show any improvement in the stabilisation of vitamin-A.

TABLE IV

Effect of antioxidants on the retention of vitamin-A and peroxide formation in a mixed sample of Khada Mushi and Waghbeer liver oils on aeration at 50° ± 5°.

	Control sample.		With Ionol (0.02%).		With propyl gallate (0.02%).	
Aeration period	Loss of vitamin-A.	Peroxide number.	Loss of vitamin-A.	Peroxide number.	Loss of vitamin-A.	Peroxide number.
32 hours	24.9%	23	15.4%	15	16.6%	12
77	30.7	34	25.9	19	27.0	12
100	39.8	43	36.0	24	33.8	25

This indicates that the mixed sample contains optimum concentration of natural antioxidants and that further addition of the antioxidants is superfluous for the stabilisation of vitamin-A.

Stability of Vitamin-A by Storage.—Samples of liver oils to which antioxidants were added were stored up to 12 months as described earlier. Most of the results as regards the efficiency of the antioxidants conform to the findings obtained with the aeration tests. Table V gives the results of storage of Khada Mushi liver oil which had an initial peroxide number of only 3 and vitamin-A content as 17,300 I.U./g. All the antioxidants except B.H.A. helped to slow down the destruction of vitamin-A up to 9 months' storage.

TABLE V

Percentage destruction of vitamin-A and peroxide formation in Khada Mushi liver oil on storage at 28° ± 2°.

	Percentage destruction of vitamin-A.			Peroxide number		
	Storage period (months)					
	7.	9.	12.	7.	9.	12.
Control sample	20.1	49.4	67.5	15	20	17
Propyl gallate (0.02%) + citric acid (0.01%)	11.6	43.4	65.8	8	12	13
D-b-*p*-cresol (0.02%)	17.2	42.3	46.0	8	14	11
B.H.A. ,,	26.8	56.1	...	17	14	...

Effect of Antioxidants on a Semi-rancid Oil

Khada Mushi liver oil (Table I) was kept at room temperature for a long time and then the antioxidants were incorporated in the usual manner. The peroxide number was high and so the concentration of the antioxidants added was also increased. The sample was stored at 38°, and vitamin-A was assayed spectrophotometrically at intervals. Along with the vilamin-A values, variations in the ratios of extinction coefficients *E* 313/*E* 328 and *E* 338.5/*E* 328 were also recorded. The initial vitamin-A content of the oil was 21,400 I.U./g. It can be observed from Table VI that the addition of antioxidants to a stored sample gives protection only for a relatively short time. After about one month, the antioxidants behave differently and give no protection to the vitamin-A present, though the concentration of the antioxidants is increased up to 0.1%. Once the peroxide formation has started in the oil, the addition of antioxidants is usually ineffective in checking the vitamin-A destruction. The increase of concentration of the antioxidants from 0.05% to 0.1% has a deleterious effect. Lundberg, Dockstader and Halvorson (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 403) observed that the increase of concentration of the antioxidants beyond 0.06% resulted in decreased stability. Kraybill, *et al.* (*ibid.*, 1949, **26**, 449) also observed that increase of concentration of B.H.A. beyond 0.06% in lard resulted in decreased stability.

TABLE VI

Percentage destruction of vitamin-A on storage at 38° ± 1°.

	Storage period (weeks)				
	2.	5 3.	8.	12.	16.
Control sample	15.0	1.8	20	23.3	40.4
Propyl gallate (0.05%)	11.7	18.0	44.6	49.6	54.9
Propyl gallate (0.1%)	0	16 1	18.2	47.0	64.5
D-b-*p*-cresol (0.05%)	2.5	...	35.8	44.2	59.4
,, (0.1%)	6.5	...	32.6	52.0	62.1
B.H.A. (0.05%)	1.4	6.0	28.2	39.4	52.7
,, (0.1%)	0	7.1	36.2	54.3	62.6

TABLE VII

Variations in the ratio E 313/E 328 for Khada Mushi liver oil on storage at 38° ± 1°.

	Storage period (weeks)				
	2.	5.3.	8.	12.	16.
Control sample	1.010	...	1.028	1.103	1.137
Propyl gallate (0.05%)	1.017	...	1.069	1.200	...
Propyl gallate (0.1)	1.079	...	1.115	1.238	1.284
D-b-*p*-cresol (0.05%)	1.075	...	1.114	1.228	1.354
,, (0.1%)	...	1.038	1.179	1.250	1.358
B. H. A. (0.05%)	1.021	1.069	1.081	1 177	1.361
,, (0.1%)	1.019	...	1.140	1.240	1.358

TABLE VIII

Variation in the ratio E 338.5/E 328 for Khada Mushi liver oil on storage at 38° ± 1°.

	Storage period (weeks)				
	2.	5.3.	8.	12.	16.
Control sample	0.8241	0.8181	0 8098	0.8041	0.7733
Propyl gallate (0.05%)	...	0.8112	0.8023	0.7550	...
Propyl gallate (0.1%)	0.8445	0.8013	0.7964	0.7532	0.7202
D-b-*p*-cresol (0.05%)	0.8243	...	0.7861	0.7526	0.7047
,, (0.1%)	...	0.8108	0.8072	0.7430	0.7082
B. H. A. (0.05%)	0.8172	0.8044	0.8015	0.7516	0 6993
,, (0.1%)	0.8204	0 8041	0.7809	0.7499	0.7068

The ratio *E* 313/*E* 328 increases, whereas *E* 338.5/*E* 328 decreases in all samples. Thus, as vitamin-A is progressively destroyed, the irrelevant absorption increases. Dassow and Stanby (*loc. cit.*) have also observed a similar trend in Halibut liver oil on aeration with the ratios *E* 300/*E* 328 and *E* 350/*E* 328.

Effect of Ultraviolet Light

Ultraviolet light destroys vitamin-A. To study the effect of addition of antioxidants against ultraviolet light, the oil (2 3 g.) with and without antioxidants was spread in petri dishes and was exposed simultaneously to ultraviolet light from a "Hanova" source at a distance of 14 inches. Table IX shows that within 2½ hours all the vitamin-A is destroyed. The oil had an initial potency of 29,500 I.U./g. vitamin-A. The added antioxidants failed to give any protection to vitamin-A. It is thus indicated that added antioxidants may have no protective action for vitamin-A in presence of direct sunlight containing ultraviolet rays.

TABLE IX

Percentage destruction of vitamin-A in Khada Mushi liver oil on exposure to ultraviolet light.

	Period of exposure (hours).			
	0.5.	1.	2.	2.5
Control sample	22.5	51.5	72.4	100
N. D. G. A. (0.02%)	26.2	51.6	75.9	100
Propyl gallate (0.02%)	29.3	55.4	71.3	100
D-b-*p*-cresol ,,	25.0	40.2	70.6	100
B. H. A. (liquid) ,,	25.7	49.6	76.1	100
,, (solid) ,,	17.6	35.0	75.7	100

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IODOMETRIC DETERMINATION OF COPPER AND IRON

By S. J. Quader and M. H. Khundkar

A new method for iodometric co-determination of copper and iron in a single aliquot is reported. This involves the determination of copper first by forming iron as the hexafluoride complex in acetic acid solution. After the titration of copper is complete, the iron complex is decomposed quantitatively with mineral acid, and the iron iodometrically determined.

Copper gives values (for Cu between 0.044 and 0.264 g.) within ± 0.4%. For successful determination of iron, the iron concentration after dilution should be below 0.56 g./litre and the concentration of sulphuric acid not less than 0.10*N*. Under these conditions up to 0.167 g. of iron could be determined in single titrations with virtually no error, but for convenience of handling the upper limit should better be kept below 0.08 g. Fe.

Iodometric determination of copper based on the reaction

$$2Cu^{++} + 4I^{-} \longrightarrow 2CuI + I_2$$

is well known. An analogous reaction takes place with ferric ion, and this interferes with the above determination of copper in a solution of their mixture. The interfering effects of ferric iron has been nullified by several earlier workers by employing suitable reagents and conditions. Very recently an iodometric estimation of both copper and iron has been reported by Brasted (*Anal. Chem.*, 1952, **24**, 1040). This work is fundamentally based on an earlier observation (Hahn and Clas, *Z. anal. Chem.*, 1929, **79**, 26) that Fe^{+++} also liberates iodine quantitatively in presence of cuprous iodide. Thus, Brasted determined the total iodine liberated by cupric and ferric ions together, and by a separate titration determined the iodine liberated by cupric ion while ferric ion had been masked by hexafluoride complex formation. His main attention was, however, focussed on avoiding interference of nitric acid by use of sulphamic acid. In has been possible in these laboratories to develop a method for iodometric co-determination of cupric and ferric ions in a single aliquot. The method is based on the following principle.

Iron hexafluoride complex is stable and ineffective towards KI in acetic acid buffered medium, but is unstable in presence of mineral acid (sulphuric acid) of suitable concentration. Thus, if to a solution containing both cupric and ferric ions (first the ferric hexafluoride complex is formed) KI is added, iodine will be liberated in quantitative equivalence of copper present. After this iodine has been titrated with a standard sodium thiosulphate, some mineral acid is added (enabling decomposition of the hexafluoride complex), when a further amount of iodine will be liberated which will be equivalent to the iron present. The present investigation deals with the conditions for a quantitative decomposition of the complex fluoride and also the limiting concentration of iron that can be employed. Results will show that under suitable conditions, this method can be employed for the quantitative determination of copper and iron in a very simple way.

EXPERIMENTAL

B.D.H. grade ferric chloride and copper sulphate were used to prepare two separate stock solutions. The ferric and copper ion contents of these were determined by known standard methods, and these results used to represent the theoretical values of the respective constituents. Known volumes of copper and iron solutions were taken and boiled with a dilute ammonia solution to precipitate iron as the hydroxide. The excess of ammonia was required to convert the copper into the amino complex. Sufficient glacial acetic acid was then added during the boil. Under these conditions, 10% ammonium fluoride solution was gradually added dropwise till all iron went into solution, care being always taken to avoid excess. The solution was then cooled, and KI solution added in excess so as to dissolve the cuprous iodide first formed. The iodine liberated was titrated with standard thiosulphate solution without starch as an indicator. It was fairly easy to determine accurately the end-point (yellow to colorless). After the titration sufficient H_2SO_4 (25 c.c. of 5*N* solution) was added and the total volume made to about 150 c.c. The liberated iodine was again titrated with thiosulphate, using starch as the indicator.

TABLE I

Titration of both copper and iron iodometrically in presence of each other.

Expt.	Amount of iron		Error.	Amount of copper		
	Calc.	Found.		Calc.	Found.	Error.
1	0.02788 g.	0.02787 g.	−0.01 mg.	0.04397 g.	0.04363 g.	−0.34mg.
2	0.02788	0.02788	−0.01	0.08794	0.08806	+0.12
3	0.02788	0.02787	−0.01	0.13191	0.13230	+0.39
4	0.02788	0.02787	−0.01	0.17588	0.17590	+0.02
5	0.02788	0.02788	Nil	0.21985	0.22020	+0.35
6	0.02788	0.02789	+0.01	0.26382	0.26410	+0.28
7	0.02788	0.02787	−0.01	0.04397	0.04363	−0.34
8	0.05576	0.05546	−0.30	0.04397	0.04363	−0.34
9	0.08364	0.08390	+0.26	0.04397	0.04396	−0.01
10	0.11152	0.10710	−4.42	0.04397	0.04426	+0.29
11	0.13940	0.13360	−5.80	0.04397	0.04396	−0.01
12	0.16728	0.14690	−20.38	0.04397	0.04363	−0.34

DISCUSSION

The amount of copper varied between 0.044 g. and 0.264 g. in the different experiments, and results were obtained within ± 0.4% of the theoretical value. This is only to be expected. In fact, there has been no material modification in the method for the determination of copper.

The amount of iron covered within this investigation was 0.028 g. to 0.167 g. For all titrations recorded in Table I, the different solutions of iron and copper were diluted to 150 c.c. and (after copper titration was over) 25 c.c. of 5.0 *N*- H_2SO_4 were added to decompose the complex. This amount of acid was found sufficient up to an original iron concentration of 0.055 g. More acid (about 35 c.c.) was required to decompose 0.084 g. of iron. For larger amounts of iron content higher proportions of the 5 *N* acid could not effect complete decomposition. In separate experiments, it was, however, noticed that if the solution was diluted to 500 c.c., and then correspondingly larger proportions of the 5*N* acid employed, accurate values for the iron content could be obtained (in so far as has been covered within this investigation). It thus occurs that for efficient titration, a concentration of iron after dilution should not exceed 0.56 g./litre, and for this an acid concentration of at least 0.10*N*- sulphuric acid is required. In order to satisfy this condition, a convenient amount of iron to be taken in single titration would be a miximum of 0.08 g. But accurate results have been obtained up to 0.167 g.

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FURTHER EXPERIMENTS ON THE ANODIC OXIDATION OF FORMATE ION

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Decomposition potentials and anode discharge potentials in the electrolysis of (1) sodium formate solutions between gold electrodes, (2) sodium formate and formic acid solutions between a copper anode and a platinum cathode, and (3) copper formate between platinum electrodes have been measured.

Anodic gases obtained at varying current densities in (1) and (3) have been analysed. Hydrogen and carbon monoxide are minor constituents in (1) at higher current densities. These products do not appear at all in (3) in which oxygen is a minor constituent at lower current densities and even this disappears completely, quite contrary to usual expectations, at high current densities when carbon dioxide becomes the sole anodic gaseous product.

These reactions are discussed in the context of the mechanisms proposed in an earlier paper (this *Journal*, 1951, **28**, 287). It has been shown that the nature and material of the electrode surface very considerably affect the values of the decomposition and discharge potentials as well as the secondary reactions at the anodes. It has been pointed out, in particular, that anodic oxygen evolution is (*a*) not due to the discharge of any ion other than formate ion, (*b*) not determined by mere voltage conditions, once electrolysis commences, in formates, and (*c*) a product of a secondary reaction involving formate radicals the extent of which reaction is determined by the material and nature of the anode.

In a previous communication (this *Journal*, 1951, **28**, 287) the authors reported the occurrence of carbon monoxide and hydrogen in the anode gases, in addition to CO_2 and O_2. Mechanisms were suggested for the formation of these products at the anode. It was suggested that formate radicals, liberated on the platinum electrode, behaved differently at different parts of the electrode and that the reactions were entirely heterogeneous. The main reaction was considered to be

$$2\,HCOO \longrightarrow HCOOH + CO_2 \qquad \ldots \quad (1)$$

At higher current densities

$$2\,HCOO + H_2O \longrightarrow 2\,HCOOH + O \qquad \ldots \quad (2)$$

At still higher current densities

$$2\,HCOO \longrightarrow CO_2 + CO + H_2O \qquad \ldots \quad (3)$$

and

$$2\,HCOO \longrightarrow H_2 + 2\,CO_2 \qquad \ldots \quad (4)$$

} minor reactions

These heterogeneous reactions may resonably be expected to vary qualitatively and quantitatively with the variation of surfaces. Experiments described in this paper were undertaken with the object of verifying this idea by (1) using gold anodes and (2) by electrolysing a stable heavy metal formate, copper formate, between platinum electrodes. The variation in the nature of electrode surface in case (2) being due to adsorption or surface contact of copper ions on the anode. These expectations have been realised as the results presented in this paper testify.

Experimental

The experimental cell for electrolysis and collection of gases was the same as the one used in the earlier work (*loc. cit.*). The other arrangements for potentiometric and other measurements were also the same as before. The anodic potential measurements were made with a saturated KCl-calomel element as the reference electrode. The values reported are after due corrections for the potential of the calomel element. The experiments were all carried out in a thermostat maintained at 30°. About 40 c.c. of anodic gases were collected in each of the experiments for analysis. Table I shows results of decomposition and anode-discharge potential measurements. Data for polished platinum anodes, from earlier work, are included for purposes of comparison.

Table I

Electrolyte.	Electrodes.	Decomposition potential.	Anode discharge potential.
N-Na formate	Polished Pt	1.555 volts	0.793 volt
,,	Gold	2.200	1.3584
N-Formic acid	Polished Pt	1.800	0.897
,,	Copper anode	0.725	0.2631
N-Formic acid	Pt cathode		
N-Na formate	Do	0.840	0.1884
N-Copper formate	Polished Pt	1.24	1 6184

The decomposition and discharge potentials depend largely on the nature of the electrode material and the above results are in support of the general finding. Experiments carried out with copper as the anode indicate a very low dissolution potential. These experiments with copper electrodes were carried out to see if at least at very high current densities there would be any gas evolution or if any breaks would be observed in current potential curves. The curves were found to be exceedingly smooth and at no stage was there any gas evolution. In fact, current efficiency measurements indicated a dissolution efficiency of more than 100%. Curves drawn from data for other electrodes also indicated only one break in each. It is noteworthy that the anode discharge potentials in sodium formate electrolysis with gold anode and in the case of copper formate electrolysis with platinum electrodes, are rather high.

Table II gives details of the findings regarding the phenomena at the anodes in the last two cases mentioned.

Discussion

Comparing the results of electrolysis of sodium formate solutions between gold electrodes with those obtained with polished platinum electrodes (*loc. cit.*), it is observed that at corresponding current densities, the anode gases from gold electrodes contain a much greater proportion of oxygen. Again, carbon monoxide is a constituent of anodic gases when current density reaches 50 milliamperes/cm^2., as was the case with

TABLE II

(1) *N*-Sodium formate.

Electrodes.	Current density in milliamp./cm².	Anode potential.	Time taken to collect gas.	Vol. of gas.	Volume % composition.			
					CO_2.	O_2.	CO.	H_2.
Gold	12.5	2.1404	6 hrs.	16 c.c.	87.5	6.5	0	6.0
	25.0	2 3302	3	17.7	82.5	10.0	0	7.50
	50 0	3.2768	1½	34.0	43.2	46.40	1.2	9.0
	125	4.4858	1 st ½ hour	41.6	25.0	66.0	1.0	8.0
			2 nd ½ ,,	45.6	32.0	60.0	1.5	6.5
	250	4.8611	10 min. in 1 st ½ hour	44.4	36.0	60.0	2.0	1 5
			10 min. in 2 nd ½ hour	50.0	38·0	58.0	2 0	2.0
			10 min. in 3 rd ½ hour	55 0	40.0	55.0	2 0	2.5c

(2) *N*-Copper formate solutions.

	12.50	1.9618	8 hrs.	6.80	90.0	10	Nil	Nil
	25.0	2.735	4	33 30	95.0	5	—	—
Platinum	50	3.6849	1	36.30	98 8	1.2	—	—
	100	3.738	20 min	44.20	100	Nil	—	—
	200	5.26	10	45.30	100	Nil	—	—

platinum anodes. But one important difference observed is that hydrogen is evolved at lesser current densities than with platinum electrodes. It therefore appears that gold surface favours the reaction

$$2\,HCOO \longrightarrow 2\,CO_2 + H_2 \qquad \ldots\ (4)$$

to a much greater extent than platinum, and even at lower surface concentrations of the free formate radicals (at lower current densities). It was observed in the case of platinum anodes that this reaction became less intensive at higher current densities. The same tendency is observable with gold electrodes also. In one important respect the reactions at gold electrodes seem to differ from those on platinum. In the case of platinum anodes, the reaction (3) yielding carbon monoxide and the reaction (4) yielding hydrogen, were both observed to commence at a current density of 12.5 milliamps./cm². and both persisted even at higher current densities. On gold, however, the reaction (4) appears at low current densities and to some extent is suppressed with the appearance of the reaction (3) at higher current densities. A third distinctive feature of the reactions on gold anodes is the larger and rather high proportions of oxygen in the anode gases at all current densities. It has long been known that higher proportions of oxygen appear only when dilute solutions are employed. As was pointed in the previous paper (*loc. cit.*), current potential studies do not indicate the discharge of more than on

species of ions on the anode, and therefore the appearance of oxygen in the anode gases should be attributed to a reaction of the formate radical itself, as depicted by equation (2) and seems to be the dominating reaction on gold electrodes at and beyond a current density of 50 milliamps./cm^2.

Copper Formate Solutions.—A very high anode-discharge potential, 1.6184 volts, is a feature of the electrolysis of this salt between platinum electrodes. The corresponding potential in the case of sodium formate is 0.793 volt (Table I). Even for an irreversible process at electrodes this difference is remarkable, because the electrode and (unless otherwise proved) the ion discharged happen to be the same in both the electrolytes. Considering the fact that the discharge potential of formate ion (from sodium formate) on gold is also quite high, 1.3584 volts, one would be led to draw the inference that copper ions adsorbed on the electrode alter the nature of the platinum surface to the extent of doubling the anode discharge potential on it. That the nature of the electrode is profoundly affected is evidenced by the reactions at the anode. In the current density range examined, which is the same as in the rest of the work hitherto dealt with, reactions (3) and (4) are not in evidence at all. Reaction (2) which crops up in all the previous experiments at current densities higher than 12.5 milliamps./cm^2. diminishes in intensity, in the case of copper formate electrolysis, with increase in current density and disappears entirely at higher current densities. This is very surprising. Oxygen evolution at the anode, in the electrolysis of formates, is not therefore determined by mere voltage conditions and considerations, but should be regarded as the product entirely of the secondary heterogeneous reaction (2) involving formate radicals, the extent of which is determined by the nature and material of the anode.

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VISCOSITY OF GLUCOSE, UREA AND GELATINE SOLUTIONS AND SOME PROPERTIES OF THE DISSOLVED PARTICLES

By J. C. Ghosh and B. P. Gyani

Viscosity of aqueous solutions of glucose, urea and gelatine has been measured at different concentrations, both by Scarpa's and Ostwald's methods. The partial specific volume (ϕ) and the shape factor (a) for the differnt solutions have been calculated from the results obtained according to the procedure of Sakurada. It is found that only gelatine at the isoelectric point gives steady values of a and ϕ. They change with the concentration in other cases and are particularly anomalous in the cases of normal gelatine and arabic acid.

A relation between the viscosity (η) of a solution and the volume concentration ϕ [= vol. of the disperse phase (solute) ÷ vol. of the solution] is given by Einstein's equation

$$\eta = \eta_0 (1 + 2.5\,\phi) \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where η_0 = viscosity of the solvent. In deducing this equation it has been assumed that the particles of the disperse phase are smooth, spherical, undeformable, and without any interaction among themselves. These conditions are seldom realised in practice, and indeed very few workers have found equation (1) to be obeyed. Further, to test eq. (1), an accurate value of the volume concentration is required, which is difficult to obtain experimentally. Under these circumstances, a modified equation

$$\eta = \eta_0 (1 + kc) \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

has been proposed, particularly by Vand (*Nature*, 1945, **155**, 364). In this equation k is a constant, and c is the volume concentration, and the equation is considered to be valid even for non-spherical particles, the factor k being a function of their shape, rigidity, and Brownian movement. For elongated particles, $k > 2.5$, tending towards 2.5 for spherical particles as in eq. (1). The nearest value so far found is 2.9 in the cases of gamboge and mastic suspensions (Bancelin, *Kolloid Z.*, 1911, **9**, 154). Sakurada (*ibid.*, 1933, **64**, 195) has discussed yet another equation

$$\eta_{sp} = \frac{ac\phi}{100} \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

where η_{sp} = specific viscosity of the solution = $\eta/\eta_0 - 1$, c = concentration of the solute in g./100 c.c., a = particle shape factor, ϕ = partial specific volume, *i. e.* the volume occupied by 1 g. of the solute in the dissolved state. Obviously, if one measures η_{sp} at different concentrations, it is possible to obtain the product $a\phi$ only, and not the individual constants separately. Sakurada therefore prefers the equation

$$\eta_{sp} = \frac{a'c\,\phi'}{100 - c\,\phi'} \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

due to Fickentscher and Mark (*ibid.*, 1929, **49**, 135) where c again = conc. in g./100 c.c., and ϕ' and a' correspond to ϕ and a in eq. (3). $c\phi'$ in the denominator is a van der Waals correction term. Eq. (4) may be written

$$c/\eta_{sp} = -c/a' + \frac{100}{a'\phi'} \quad \ldots \quad \ldots \quad \ldots \quad (5)$$

which is the equation of a straight line between c/η_{sp} and c, from the plot of which $-1/a'$ and $100/a'\phi'$ may be readily determined, yielding individual values of a' and ϕ'. If the specific viscosity is η_{sp_1} at concentration c_1 and η_{sp_2} at conc. c_2, one obtains the equations,

$$c_1/\eta_{sp_1} = \frac{c_1}{a'} + \frac{100}{a'\phi'}; \text{ and}$$

$$c_2/\eta_{sp_2} = -c_2/a' + \frac{100}{a'\phi'}$$

from which one can readily find that

$$\phi' = \frac{100\,(\eta_{sp_2}/c_2 - \eta_{sp_1}/c_1)}{\eta_{sp_2} - \eta_{sp_1}}; \text{ and}$$

$$a' = \frac{c_2 - c_1}{c_1/\eta_{sp_2} - c_2/\eta_{sp_2}}.$$

Eisenschitz (*Z. physikal. Chem.*, 1931, **A 158**, 78) expresses the relation between the length and diameter of the particles in terms of η_{sp}, ϕ, and c by means of the equation

$$\eta_{sp} = \left\{ 0.159\, l/d.\, \frac{1}{\log 2l/d} \right\} \frac{\phi c}{100}$$

so that this equation is of the same form as that of Sukurada, the value of a in eq. (3) being

$$= 0.159\, l/d.\, \frac{1}{\log 2l/d}.$$

Equation (5) has been applied by Sakurada in the cases of squalene, polysterol, cellulose, nitrocellulose and rubber with success (cited by Hollihan and Briggs, *J. Phys. Chem.*, 1942, **46**, 685). Theis and Bull (*ibid.*, 1936, **40**, 125) also applied equation (5) to a solution of stearic acid in CCl_4 and found values of a' and ϕ' in agreement with those of other workers. However, Hollihan and Briggs (*loc. cit.*) studied the same solutions and obtained a higher value for a'. In an attempt to explain the apparent anomaly they measured the values of a' and ϕ' at different air pressures applied upon the column of liquid and observed that the values of a' (and hence the length/diameter ratio) decreased with increase in air pressure. They concluded from these results that 'Sukurada's equation is a mathematical artifice'.

It must be said that Sukurada's method is an easy one for finding a' and ϕ'. It is curious, as Hollihan and Briggs also note (*loc. cit.*) that no notable attempts have been made in this direction. As Sakurada finds, his method is applicable in a number of cases, and as there is an apparent controversy regarding its applicability, it is worthwhile to pursue the method further.

EXPERIMENTAL

It is necessary to employ an accurate method of measuring viscosity for this investigation. The relative merits of the methods due to Farrow and Ostwald have been discussed in a previous communication (this *Journal*, 1952, **29**, 615). Although the former is on the whole more convenient, Chatterjee and Vaish point out (*ibid.*, 1935, **12**, 6) that Farrow's method does not give good results with true solutions. Under these circumstances, we have made the measurements using both the methods, and find that the results agree within 0.5%. Ostwald's viscometer used by us has a capacity of 20 c. c., and takes about 125 sec. at 35° for water to flow out. In the other apparatus we have further increased the size of the air reservoir, thus securing a better constancy of the suction pressure (Farrow's method).

Glucose, urea, gelatine and gum acacia solutions in water were investigated. The concentration ranges were made as large as possible. Urea and glucose were of A. R. quality and were not purified further. The gelatine was purified according to Field (*J. Amer. Chem. Soc.*, 1921, **43**, 67). The gum acacia was a good sample from the British Drug House Ltd. These samples were all dried over P_2O_5 for two days before weighing out for preparing stock solutions with air-free water. The solutions were stocked in resistant glass bottles and the measurements were carried out 24 hours after the preparation of the solutions in each case so that differences due to ageing of the solutions were largely eliminated. The temperature was throughout maintained at 35° ± 0.1°. The periods of flow in no case differed by more than 0.2 sec. for the same solution. Two or three different suction pressures were used in Farrow's method. The individual results agreed well with each other as can be seen from Table I.

TABLE I

Viscosity of urea soln. (7.5 g./100 c.c.) *by Farrow's method at diff. suctions.*

Suction. (cm. water)	t_1.	t_2	$\left(\frac{t_1 t_2}{t_1+t_2}\right)_s = y$.	$\left(\frac{t_1 t_2}{t_1+t_2}\right)_w = x$.	$y/x = \eta_r$.	Mean η_r.	η_{sp}.
45	22.80 sec.	119.78 sec.	19.154	18.265	1.0487		
35	30.65	119.90	24.410	23.187	1.0527	1.0511	0.0511
25	47.46	119.87	33.999	32.323	1.0519		

The table shows that the extreme values of η_r differ from each other by about 0.3%. In no case was a difference larger than 0.5% observed. Table II contains the results of measurements with other solutions some of which have been taken from other workers.

TABLE II

Viscosity of solutions at diff. conc. at 35°.

Conc. (g./100 c.c.)	η_r mean.	$\eta_{sp.}$	c/η_{sp}	ϕ' calc.	a' calc.	$a'\phi'$.
		A. Urea (Scarpa's or Farrow's method).				
7.5	1.0511	0.0511	146.7	1.942	0.300	0.5826
15.0	1.1232	0.1232	121.7	1.006	0.700	0.7042
22.5	1.2027	0.2027	111.0	0.944	0.728	0 6872
30.0	1.2980	0.2080	100.7	...	...	...
		B. Glucose (Scarpa's method).				
4.80	1.1185	0.1185	40.50	1.80	1.22	2 192
9 60	1.2625	0.2625	36,57	2.01	1.10	2.211
14.40	1.4470	0.4470	32.21	1.57	1.53	2.402
25.56	2.0256	1.0256	24.92	1.21	2.30	2.793
30.00	2.3050	1.3050	22.99	...	...	...
C. Gelatine at p_H 8.0 at 40° (calc from the data of Davies and Oakes, *J. Amer. Chem Soc.*, 1922, **44**, 464) (Ostwald's method).						
1.00	1.524	0.524	1.908	18.7	2.272	42.486
2.00	2.362	1.362	1.468	15.2	3.125	47.500
3.00	3.612	2.612	1.148	11 9	4.651	55.347
4.00	5.289	4.289	0.933	12.4	4 310	53.444
5.00	8.125	7.125	0.701	...	...	...
		D. Gelatine solutions at p_H 4.7 at 35° (Scarpa's method).				
0.3	1.0525	0.0525	5.72	21.18	0.769	16.28
1.0	1.2076	0.2076	4.81	21.28	0.714	15.19
1.5	1.3645	0.3645	4.11			
E. Normal gelatine at 35° (calc. from the data of Bogue, *ibid.*, 1921, **43**, 1764) (Ostwald's method).						
0.05	1.023	0.023	2.17			
0.10	1.045	0.045	2.22			
0.20	1.073	0.073	2.74			
0.40	1.124	0.124	3.22			
0.60	1.211	0.211	2.84			
0.80	1.328	0.328	2.44			
1.00	1.404	0.404	2.47			
1.50	1.522	0.522	2.87			
2.00	1.818	0.818	2.44	20.61	1.162	23.948
2.50	2.100	1.100	2.27	10.99	2.941	32.322
3 00	2.400	1.400	2.14	9.00	3.846	34.614
3.50	2.740	1.740	2.01	8.82	3.846	33.922
4.00	3.078	2.078	1.92	6.51	5.555	36.163
		F. Gum acacia (Ostwald's method).				
3.99	3.6182	2.6182	1.521			
3.22	3.1088	2.1088	1.527	0.510	127.67	65.11
2.10	2.4292	1.4292	1.470	3.335	19.63	65.85
1.06	1.7682	0.7682	1.379	6.793	11.44	77.72
0.45	1 3783	0.3783	1.200	27.751	3.39	94.95

TABLE II (*contd.*)

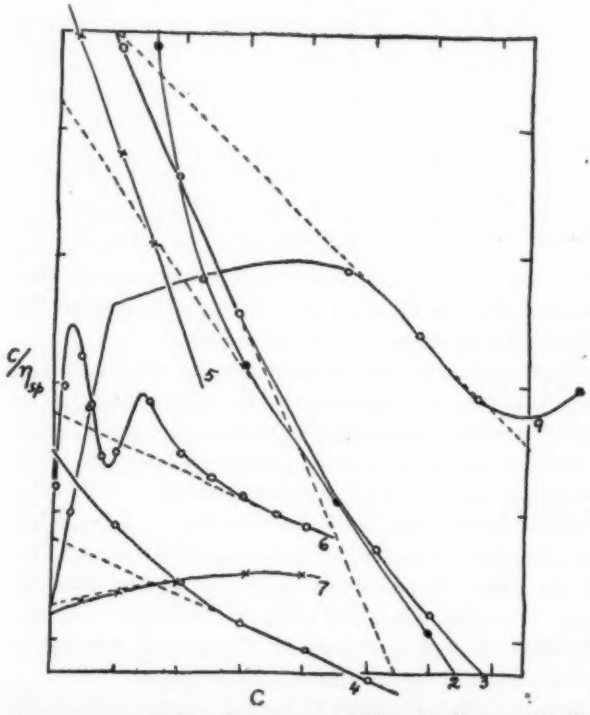
G. Arabic acid solutions at 35° (calc. from Mukherjee and Bhowmik's data, this *Journal*, 1949, **26**, 151) (Ostwald's method).

Conc.	η_r mean.	η_{sp}.	c/η_{sp}.	ϕ' cal.	a' calc.
0.028	1.15	0.15	0.1866		
0.057	1 25	0.25	0.2280	−2350	−0.700
0.115	1.37	0.37	0 3108	−9733	−0 700
0.230	1.67	0.67	0.3433	−1016	−3.538
0.460	2 40	1.40	0.3286	2079	15.542
0.575	2 90	1 90	0 3026	5200	4.444
0.670	3 40	2 40	0.2791	5580	4 042
0.850	4.32	3.32	0.2560	3511	7.792
0.920	5.12	4.12	0.2233	7150	2.141

H. Arabic acid at 45° (calc. from the data of Mukherjee and Bhowmik, *loc cit.*) (Ostwald's method).

0 028	1.14	0.14	0.2400	$-24\,85\times10^3$	−0.341
0.057	1.20	0 20	0 2850	− 7.30 ,,	−0.681
0.115	1.31	0.31	0.3790	− 0.30 ,,	−9.200
0.230	1.60	0.60	0.3833	− 0 11 ,,	−23.499
0.460	2.17	1.17	0 3931	0.74 ,,	2.264
0.575	2.68	1.68	0 3423	0.84 ,,	1.816
0.670	3.31	2.31	0.2900	0.18 ,,	21.95
0 850	3.85	2.85	0.2982	0.72 ,,	1.79
0.920	4.55	3.55	0.2591		

FIG. 1



DISCUSSION

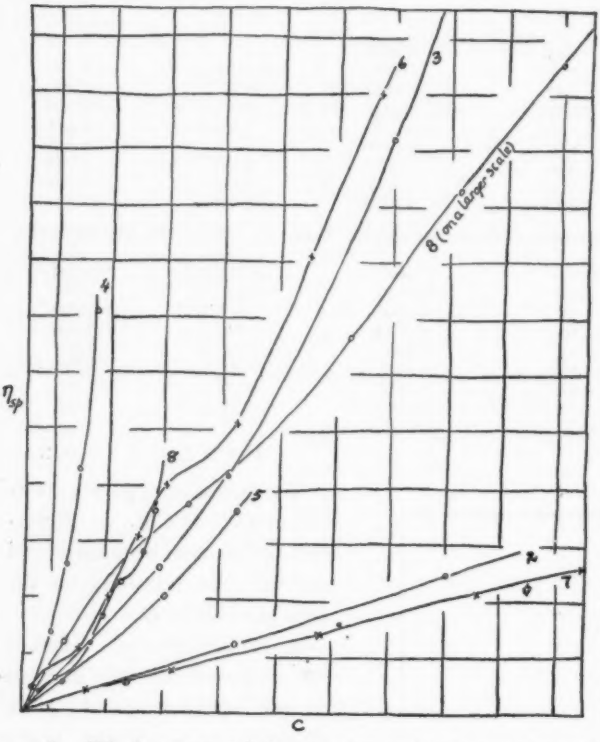
The values of c/η_{sp} are plotted against c in Fig. 1. Different scales have been employed for different solutions for the sake of convenience.

A study of the different curves shows that only gelatine at the isoelectric point gives a straight line, *i.e.* a' and ϕ' are constant. Normal gelatine and arabic acid give very anomalous curves. They show distinct periodicity at lower concentrations, and the values of a' and ϕ' tend to become often negative, resulting from a positive slope. The curve for gum acacia also starts off with a small positive slope at lower concentrations, but this curve possesses no periodicity.

When a' is positive it increases with concentration in most cases. This will mean according to Eisenschitz (*loc. cit.*) that the length/diameter ratio of the particles are increasing or the shape of the dissolved particles is departing

more from that of a sphere as the solutions become more and more concentrated. A simple proportionality will be disturbed if (*i*) there is increased Brownian movement with larger number of solute particles present, (*ii*) there is a tendency towards some kind of regular arrangement of the particles, (*iii*) there is a van der Waals attraction between them and (*iv*) the solute particles are polydisperse. Perhaps (*i*) and (*iv*) are absent in the case of glucose and urea, but even in these cases (*ii*) and (*iii*) are operative when the solution is concentrated. Since solutions as concentrated as 30% have been used in Tables IIA and IIB, the non-constancy of a' values is not too difficult to understand. What is curious is that with normal gelatine, gum acacia and arabic acid, we get negative values for a' and also for ϕ'. These values are shown in columns 5 and 6 in the above tables. A negative value of ϕ' will mean that the van der Waals correction is negative and it is not clear what physical meaning could that have. These observations lead one to doubt, as Hollihan and Briggs did, whether the constants of the equation used by Sakurada have any physical significance.

FIG. 2



If equation (3) be correct, the $\eta_{sp} - c$ curves should pass through the origin. These curves are shown in Fig. 2, and are drawn on different co-ordinates according to convenience. In most cases one obtains a smooth curve with slight convexity towards the concentration axis, but normal gelatine and arabic acid have points of inflexion. It is unlikely that these breaks could be due to changes in hydration or structure. Mukherjee and Bhowmik (*loc. cit.*) ascribed such a break with an arabic acid sol to aggregation but since the sol was polydisperse, it is not quite clear what they meant by aggregation effect. It appeares therefore that the cause of periodicity in $\eta_{sp} - c$ graphs must at present remain an open question. It may be noted that not only concentration of the disperse phase but also the concentration of hydrogen ions in the system determines periodicity, as is apparent from a comparison of the different graphs for gelatine.

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STUDIES IN THE CLAISEN REARRANGEMENT. PART I. REARRANGEMENT OF ALLYL-*p*-*tert*.-BUTYLPHENYL ETHER

A. B. SEN AND R. P. RASTOGI

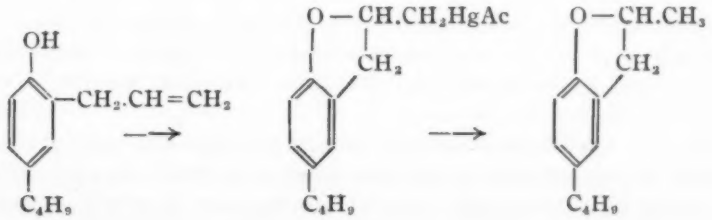
Allyl-*p*-*tert*.-butylphenyl ether has been thermally rearranged to *o*-allyl-*p*-*tert*.-butylphenol. Various derivatives of the latter compound have been described.

On thermal treatment, allyl ethers of phenols are found to rearrange to the corresponding *o*-substituted phenols (Claisen, *Ber.*, 1912, **45**, 3157). If the *o*-positions are not free, the allyl group migrates to the *p*-position. A considerable amount of work has been done in this connection by various authors (Tarbell, "Organic Reactions", 1944, Vol. II, p. 1), but little attention has been paid to the rearrangement of *p*-substituted alkyl phenylallyl ethers. It was therefore considered of interest to undertake a study of the rearrangement of the allyl ether of *p*-*tert*.-butylphenol and some of its derivtaives which might prove of interest in organic syntheses.

Allyl-*p*-*tert*.-butylphenyl ether rearranged smoothly on thermal treatment, to give a 83% yield of *o*-allyl-*p*-*tert*.-butylphenol (I) which was characterised through its 3: 5-dinitrobenzoate and the carboxymethyl ether derivatives. The latter was prepared according to the method of Koelsch (*J. Amer. Chem. Soc.*, 1931, **53**, 304).

Oxidation of the allylphenol with $KMnO_4$ in acetone solution gave polymerised, orange coloured, amorphous powders which could not be purified. However, the methyl ether on oxidation gave the expected 2-methoxy-5-*tert*.-butylphenylacetic acid and a crystalline neutral product which analysed for $C_{10}H_{16}O_2$. This product has not been identified so far. The allylphenol was catalytically hydrogenated to the propyl derivative in 80% yield.

The allylphenol on heating with KOH isomerises to the propenyl derivative which has been characterised through m.p. and mixed m.p. of the corresponding carboxymethyl ethers of the allyl- and propenylphenols. A higher boiling fraction was also obtained during the isomerisation which had an indefinite distilling range, 150°-220°/2.5 mm., and appeared to be, on the basis of the M.W. estimation, a mixture of higher polymers.



(I) (II) (III)

On heating the allylphenol with pyridine hydrochloride or hydrobromic acid in acetic acid solution, ring-closure took place and 2-methyl-2:3-dihydro-*p-tert.*-butylbenzofuran (III) was obtained in 72% yield. It gives an intense violet dye with a solution of isatin in concentrated sulphuric acid. The structure of 2-methyl-2:3-dihydrobenzofuran was finally confirmed by its synthesis through the mercurated dihydrobenzofuran according to Admas *et al.* (*J. Amer. Chem. Soc.*, 1922, **44**, 1781).

EXPERIMENTAL

Allyl-p-tert.-*butylphenyl Ether.*—*p-tert.*-Butylphenol (10 g.), allyl bromide (10 g.) and anhydrous potassium carbonate (12 g.) were refluxed in dry acetone (30 c.c.), with stirring, for 8 hours. Most of the solvent was then distilled off, water (100 c.c.) was added to the reaction mixture, and it was extracted repeatedly with petroleum ether, the extract was washed with dilute alkali, followed by water and dried over anhydrous sodium sulphate and then the solvent removed. The residual oil was distilled under reduced pressure, b.p. 115°-116°/3 mm., yield 10.5 g., n_D^{28}, 1.5065. (Found: C, 81.9; H, 9.6. $C_{13}H_{18}O$ requires C, 82.1; H, 9.4 per cent).

Rearrangmeent of Allyl-p-tert.-*butylphenyl Ether.*—The allyl ether (9.8 g.) was heated at 210°-220° for 1½ hours in an atmosphere of carbon dioxide. (The temperature rose to 238° due to the exothermic nature of the rearrangement). The reaction product was cooled and dissolved in petroleum ether and extracted with 15% caustic soda solution. The alkaline layer was worked up in the usual manner, and it gave an oily residue which distilled at 125°-126°/5 mm., yield 7.8 g., n_D^{28}, 1.522. (Found: C, 82.0; H, 9.6. $C_{13}H_{18}O$ requires C, 82.1; 9.4 per cent).

3:5-*Dinitrobenzoate of* o-*Allyl*-p-tert.-*butylphenol.*—3:5-Dinitrobenzoyl chloride (1 g.) was added to the allylphenol (1 g.) in dry benzene and refluxed for 4 hours. The dinitrobenzoate crystallised out from hot alcohol as straw-coloured needles, m.p. 84°, yield 0.85 g. (Found: N, 7.3. $C_{20}H_{20}O_6N_2$ requires N, 7.2 per cent).

o-*Allyl*-p-tert.-*butylphenylcarboxymethyl Ether.*—To a solution of the allylphenol (0.5 g.) in 33% aqueous caustic soda (2 c.c.), 50% monochloroacetic acid (1.4 c.c.) was added and the reaction mixture was heated on a water-bath for 2 hours. After cooling, the mixture was acidified and extracted with ether. The ethereal layer was shaken with 5% sodium bicarbonate solution which was acidified and extracted with ether. The ether fraction gave a sticky residue which crystallised from petroleum ether, m.p. 115°, yield 0.23 g. (Found: C, 72.6; H, 8.0. $C_{15}H_{20}O_3$ requires C, 72.5; H, 8.1 per cent).

o-*Allyl*-p-tert.-*butylphenylmethyl Ether.*—Methylation of the allylphenol (5 g.) with dimethyl sulphate (8 c.c.) in aqueous caustic soda (20%) gave the methyl ether which distilled at 97°-100°/2 mm., yield 4.6 g., n_D^{34}, 1.5042. (Found: C, 82.6; H, 10.0. $C_{14}H_{20}O$ requires C, 82.3; H, 9.8 per cent).

Oxidation of o-*Allyl*-p-tert.-*butylphenylmethyl Ether.*—The methyl ether (1.7 g.) was dissolved in acetone and an aqueous solution of $KMnO_4$ (2.1 g.) was added to it during a period of 1 hour, with constant stirring, and then left overnight at room temperature. The precipitated manganese dioxide was removed with sulphurous acid, most of the acetone distilled off and the clear solution extracted with ether. The

ethereal layer was shaken with dilute sodium bicarbonate solution which gave an acidic residue on working up in the usual manner. This residue was purified through benzene-petroleum ether precipitation and the resultant solution concentrated, made turbid with petrol ether and kept in the refrigerator. A deposit of colorless needles of 2-methoxy-5-*tert.*-butylphenylacetic acid was thus obtained, m.p. 113°, yield 0.15 g.. (Found : Neut. equiv., 218. $C_{13}H_{18}O_3$ requires neut. equiv., 222).

The neutral ethereal layer, from the above, was washed free of alkali, dried and freed of the solvent. It was dissolved in petroleum ether and kept in cold, when colorless shining plates were obtained, m.p. 84°, yield 0.2 g. It does not yield an acetyl or *p*- nitrobenzoyl derivative or a semicarbazone. On oxidation of this substance with $KMnO_4$, no acid component could be isolated and the original product was obtained back. [Found : C, 71.3 ; H, 9.2 ; M.W. (Rast), 161. $C_{10}H_{16}O_2$ requires C, 71.4 ; H, 9.5 per cent. M.W., 168].

o-*Propyl*-p-tert.-*butylphenol.*—*o*-Allyl-*p*-*tert.*-butylphenol (2 g.) in methanolic solution was shaken with platinum black (0.1 g.) in an atmosphere of hydrogen, 274 c.c. of which were absorbed at 27.5°/751.6 mm. The reaction product was filtered and the methanol removed. The residue on fractionation gave the propyl derivative distilling at 99°-103°/1 mm., yield 1.6 g., n_D^{35}, 1.504. (Found : C, 80.8 ; H, 10.5. $C_{13}H_{20}O$ requires C, 81.2 ; H, 10.4 per cent).

Isomerisation of o-*Allyl*-p-tert.-*butylphenol to* o-*Propenyl*-p-tert.-*butylphenol.*—*o*-Allyl-*p*-*tert.*-butylphenol (5 g.) and caustic soda (10 g.) in water (5 c.c.) were heated together at 170° for an hour. The reaction mixture was diluted with water to 50 c.c., acidified, and extracted with ether. The ethereal solution yielded a residue which was fractionally distilled twice and the following fractions were obtained :

(1) 120°-126°/2.5 mm. (0.5 g.). It is mainly the unchanged allylphenol.

(2) 129°-134°/2.5 mm. (3.2 g.). It is the propenyl derivative, n_D^{28}, 1.5355. (Found : C,81.75; H, 9.02. $C_{13}H_{18}O$ requires C, 82.1 ; H, 9.4 per cent).

(3) 150°-220°/2.5 mm. (1.1 g.). It is a semi-solid, sticky mass, M.W. (Rast), 458.

o-*Propenyl*-p-tert.-*butylphenylcarboxymethyl Ether.*—The propenylphenol (0.5 g.), from the fraction (2) above, gave, according to the procedure outlined earlier, the carboxymethyl ether as colorless needles from petroleum ether solution, m.p. 101°, yield 0.12 g. (Found : C, 72.4 ; H, 8.1. $C_{15}H_{20}O_3$ requires C, 72.5 ; H, 8.1 per cent). On admixture with *o*-allyl-*p*-*tert.*-butylphenylcarboxymethyl ether, it melts at 88-93°.

2-*Methyl*-2:3-*dihydro*-p-tert.-*butylbenzofuran* (III) : (*a*) *Cyclisation with Pyridine Hydrochloride.*—The allylphenol (5 g.) and pyridine hydrochloride (3 g.) were heated at 220°-225° for 2 hours in an oil-bath. The mixture was diluted with water and extracted with ether. The ethereal solution, after being washed free of phenol, gave an oily residue which was fractionated under reduced pressure and the following fractions were collected :

(*i*) 106°-107°/2 mm. (3.6 g.), n_D^{35}, 1.512. It gives a deep violet dye on treatment with isatin in concentrated sulphuric acid and does not absorb bromine in carbon tetrachloride solution. (Found: C, 82.0; H, 9.4. $C_{13}H_{18}O$ requires C, 82.1; H 9.4 per cent).

(*ii*) 150°-210°/2 mm. (1.2 g.). It is a pale, viscid mass and does not absorb bromine in carbon tetrachloride solution. M.W. (Rast), 288.

(*b*) *Cyclisation with Hydrobromic Acid in Acetic Acid.*—The allylphenol (5 g.) on treatment with 50% hydrobromic acid in acetic acid (24 c.c.), under conditions outlined above, gave identical results.

2-*Acetoxymercurimethyl*-2:3-*dihydro*-p-tert.-*butylbenzofuran* (II).— *o*-Allyl-*p*-*tert*.-butylphenol (I) (5 g.) was added slowly to 100 c.c. of aqueous mercuric acetate (9 g.) solution with stirring which was continued for 4 hours. The mercurated derivative separated out as a thick oil, which solidified on cooling and was crystallised from dilute alcohol as colorless needles, m.p. 98°, yield 11.5 g. (Found : Hg, 46.6. $C_{15}H_{20}O_2Hg$ requires Hg, 46.0 per cent).

2-*Iodomercurimethyl*-2:3-*dihydro*-p-tert.-*butylbenzofuran*.—To a solution of acetoxymercurimethyl derivative (II) (10 g.) in methanol (50 c.c.), was added 100 c.c. of aqueous potassium iodide (9 g.), with stiring and after the addition, it was warmed on a water-bath for 1½ hours. On removal of methanol, a semi-solid separated out which solidified and crystallised from alcohol, m.p. $13_4°$, yield 5.0 g. (Found : Hg, 40.5. $C_{13}H_{17}O$. HgI requires Hg, 40.0 per cent).

2-*Methyl*-2: 3-*dihydro*-p-tert.-*butylbenzofuran* (III).—A suspension of 2-iodomercurimethyl derivative (4 g.), potassium iodide (4 g.) and iodine (2 g.) in water (100 c.c.) was stirred and heated to boiling over a small flame for an hour during which the colour of iodine was discharged. The flame was removed and stirring continued for another half an hour. The reaction mixture was then extracted with ethyl acetate and the dark oily residue, obtained from the ethyl acetate layer, was heated in vacuo at 100° for 1 hour to remove as much iodine as possible, and then distilled. 2-Iodomethyl derivative was obtained as a colorless liquid, b.p. 147°-150°/1 mm., yield 2.9 g.

The above derivative was heated on a water-bath with granulated zinc (5 g.) and 25% hydrochloric acid (30 c.c.) for 3 hours with stirring. The residue, obtained through ethereal extraction, was distilled in vacuo, , b.p. 90°-92°/1 mm., yield 1.8 g., n_D^{35}, 1.513. It gives an intense violet dye with isatin in concentrated sulphuric acid. (Found : C, 81.8 ; H, 9.5. $C_{13}H_{18}O$ requires C, 82.1 ; H, 9.4 per cent).

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PREPARATION OF ANTIBACTERIALS FROM ORGANO-MERCURIALS. PART VII. CHLOROMERCURI-AMINES AND -PHENOLS

By S. S. Guha Sircar and Mahendra Kumar Rout

Preparations of chloromercuri-amines and -phenols by modified methods and their antibacterial actions have been studied. It may be concluded that replacement of the acetoxymercuri group by chloromercuri group augments the bactericidal action to a marked degree.

The present paper deals with a systematic study of the preparation and bactericidal properties of a large number of chloromercuri-amines and -phenols, since a few of them had been found earlier to be more effective than the corresponding acetoxymercuri derivatives (Guha Sircar and Mitra, this *Journal*, 1951, **28**, 80).

Chloromercuri compounds have been prepared in several ways by different workers. Doses quelle (*Bull. soc. chim.*, 1894, **11**, 263) prepared *p*-chloromercuri-phenol by interaction of K phenolate and $HgCl_2$. Dimroth (*Ber.*, 1898, **31**, 2155; 1899, **32**, 761) obtained it by addition of a saturated solution of NaCl to the hot filtrate from the interaction of HgO, glacial acetic acid and phenol in hot aqueous solution. Neogi and Mukherjee (this *Journal*, 1935, **12**, 211) prepared chloromercuri compounds of thymol, naphthol, carvacrol, nitrophenol, 8-hydroxyquinoline and nitroanilines by adding the mercuri compounds to a mixture of $NaHCO_3$ and $HgCl_2$ in presence of glycerine. The chloromercuri derivatives of aromatic amines prepared in this way have been reported to be N-Hg compounds. In the case of dihydric and trihydric phenols, oxymercuri chlorides were, however, formed (this *Journal*, 1928, **5**, 221).

Experimental

In the present work, chloromercuri-phenols were prepared by adding an excess of dilute HCl to an alkaline solution of the acetoxymercuri-phenol. Chloromercuri-amines were prepared in two ways: (*i*) by adding a concentrated solution of NaCl to a solution of acetoxymercuri-amines in acetic acid and (2) by a method, modelled on Bruice's method (*J. Amer. Chem. Soc.*, 1950, **72**, 598) for the preparation of acetoxymercuri compound. Instead of isolating the acetoxymercuri compound and then adding NaCl to its acetic acid solution, NaCl was added to the reaction mixture from which the acetoxymercuri compound was to have separated. Better yields were obtained by the second method. As expected (cf. Part VI of this series, this *Journal*, 1952, **29**, 779), Bruice's method failed in the case of *o*-chloroaniline and gave very poor yields in case of *o*-, *m*- and *p*-nitroanilines. In these cases, the reaction mixture was therefore kept at a lower p_H (between 2 and 3) before adding NaCl in order to get better yields.

Estimation of mercury and the comparative bactericidal action were made as in the previous parts (*loc. cit.*).

The properties and analyses of the compounds, not known already, are given in Table I.

TABLE I

Name of the compound.	% Hg. Found.	% Hg. Calc.	% Cl. Found.	% Cl. Calc.	M.p.
1. *p*-*R-*m*-chloroaniline	54.8	55.3	—	—	130° (d)
2. 2 :4-Di-R-α-naphthylamine	65.0	65.4	11.4	11.60	120° (d)
3. 1-R-β-naphthylamine.	52 6	52.9	9.0	9.39	170°
4. R-*o*-anisidine	55.6	55.9	9.6	9.92	197°
5. R-benzidine	48.0	47.7	8.6	8.48	208°
6. R-carvacrol	52.5	52.9	9.5	9.30	176°
7. R-*p*-hydroxybenzoic acid	53.4	53.6	9.2	9.50	Above 250°
8. R-thiosalicylic acid	51.0	51.0	9.5	9.20	,, ,,
9. R-resorcylic acid	52.0	51.6	9.0	9.20	,, ,,
10. R-vanillin	51.0	51.6	9.3	9.20	134°

*R=chloromercuri group.

The results of the yields by the two methods of preparation along with those of biological tests indicating the maximum effective dilution (M.E.D.) of the mercurials at 10 minutes' contact have been given below. The temperature was 35°. The test organisms were 24 hours' cultures of *E. Coli* and *Staphylococcus aureus*.

TABLE II

Name of the compound.	% Yields with repect to acetate. 1st method.	2nd method.	M. E. D. E. Coli.	Staphylococcus.
1. * R-aniline	53	—	1 :75,000	1 :25,000
2. R-*o*-toluidine	—	94	1 :75,000	1 :25,000
3. R-*m*-toluidine	43	92	1 :60,000	1 :20,000
4. R-*p*-toluidine	50	94	1 :60,000	1 :20,000
5. R-*o*-chloroaniline	57	Unsuccessful	1 :150,000	1 :50,000
6. R-*m*-chloroaniline	50	92	1 :150,000	1 :25,000
7. R-*p*-chloroaniline	53	93	1 :150,000	1 :50,000
8. R-*o*-nitroaniline	60	10	1 : 8,000	1 :10,000
9. R-*m*-nitroaniline	57	11	1 : 8,000	1 :12,000
10. R-*p*-nitroaniline	64	13	1 :10,000	1 :10,000
11. 2:4-Di-R α-naphthylamine	Unsuccessful	92	1 :150,000	1 :60,000
12. 1-R-β-naphthylamine	Do	94	1 :125,000	1 :20,000
13. *p*-R-*o*-anisidine	—	71	1 :150,000	1 :30,000
14. *p*-R-methylaniline		71	1 :150,000	1 :60,000
15. *p*-R-dimethylaniline		69	1 :200,000	1 :60,000
16. *p*-R-benzidine		70	1 :100,000	1 :20,000
17. *p*-R-phenol	90		1 :12 000	1 :25,000
18. *p*-R-*o*-cresol	94		1 :15,000	1 :12,000
19. *p*-R-*m*-cresol	98		1 :18,000	1 :20,000
20. *o*-R-*p*-cresol	99		1 :20,000	1 :16,000
21. *o*-R-*p*-nitrophenol	99		1 : 4,000	1 : 2,000
22. *p*-R-thymol	90		1 :12,000	1 :15,000
23. *p*-R-carvacrol	99		1 :10,000	1 : 6,000
24. 1-R-β-naphthol	94		1 :30,000	1 :15,000
25. 3-R-salicylic acid	91		1 : 4,000	1 : 2,000
26. 2-R-*p*-hydroxybenzoic acid	85		1 : 4,000	1 : 2,000
27. R-resorcylic acid	80		1 : 4,000	1 : 2,000
28 R-thiosalicylic acid	90		1 : 4,000	1 : 2,000
29. *p*-R-vanillin	90		1 : 8,000	1 : 4,000
30. *o*-R-*p*-chlorophenol	94		1 :20,000	1 :15,000
31 *p*-R-*o*-nitrophenol	90		1 : 4,000	1 : 2,000

* R=chloromercuri group.

The replacement of the acetoxymercuri group by chloromercuri group has been found to augment the bactericidal action to a marked degree. This fact can be brought out clearly by considering the case of aniline. The M. E. D. s of acetoxymercuri-aniline (Part VI, *loc. cit.*) are 1:400 and 1:1000 in case of E. Coli and staphylococcus, respectively. These activities attain values of 1:75,000 and 1:25,000, respectively, after substitution of chloromercuri group for acetoxymercuri group. The increase is not so marked in other cases. As observed in the case of acetoxymercuri-amines and phenols (cf. Parts V and VI) negative groups like COOH and NO_2 reduce activities (compounds 8, 9, 10, 21, 25, 26, 27, 28, 31). The activities of chloromercuri-α- and -β-naphthylamines, chloroanilines, mono and dialkyl anilines (compounds 5, 6, 7, 11, 12, 14, 15) are quite high, being near about 1:150,000 to 1:200, 000.

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PREPARATION OF ANTIBACTERIALS FROM ORGANO-MERCURIALS. PART VIII. MERCURATED AZO DYES DERIVED FROM DIAZOTISED ANILINE

BY S. S. GUHA SIRCAR AND MAHENDRA KUMAR ROUT

Preparation and antibacterial action of mercurated azo dyes derived from diazotised aniline have been described. The maximum activity noted in the series was 1 : 450,000. Dyes prepared by coupling with mercurated chloroanilines and α- and β- naphthylamines are more active than those prepared from other amines and phenols.

Dyes belonging to different groups are used as antiseptics, chemotherapeutic agents and for specific effects upon tissue cells in modern therapy. Quite prominent among these are some azo dyes such as Scarlet Red, Dimazon, Pyridium, Chloroazodin, Prontosil, Rubiazol and Azo-sulfamide.

Mercury also possesses intrinsic antibacterial action to a pronounced extent. The introduction of Hg in various aromatic molecules in the form of mono- or di-acetoxymercuri groups has been found to greatly augment the bactericidal action (cf. this *Journal*, 1950, **27**, 535 ; 1951, **28**, 80, 83).

The bactericidal effects of a few mercurated azo dyes were first studied by Proskouriakoff and Raiziss (*J. Amer. Chem. Soc.*, 1925, **47**, 1974) who found that they were less powerful than $HgCl_2$ but were more powerful than mercurochrome. These studies do not enable one to draw conclusions regarding the influence of different groups and nuclei on the activities of the dyes, nor do these azo dyes actually possess high bactericidal potency.

In the present paper, mercurated azo dyes having the following structural characteristics have been studied :

(1) One or more acetoxymercuri group in the diazotised half without there being any Hg in the coupled half.

(2) Naphthalene nucleus in the coupled half.

(3) One or two of such groups as OH, NO_2, COOH, SO_3H, NH_2 in different positions of the benzenoid or naphthalene nucleus of the coupled half.

EXPERIMENTAL

Mercurated azo dyes have been prepared by (*i*) the coupling of diazo solutions with mercurated phenols (Dimroth, *Ber*, 1902, **35**, 2859 ; *Chem. Zentrl.*, 1901, I, 452) and (*ii*) the coupling of diazotised mercurated amines with substances such as dimethylaniline (Jacobs and Heidelberger, *J. Biol. Chem.*, 1915, **20**, 516).

Aniline was diazotised in acetic acid medium and was then coupled with the reagents given in parenthesis in column 1 in Table I. While coupling with 2: 6-diacetoxymercuri-*p*-cresol and 2: 4-diacetoxymercuri-α-naphthol, one of the two acetoxymercuri groups was knocked off and the coupling took place in the positions thus rendered vacant (compounds 19, 23).

All the experimental data are summarised in Table I, along with the results of biological tests made in the same manner as described in earlier communications.

No marked improvement in bactericidal activity was found in azo dyes obtained from mercurated amines and phenols. Dyes prepared by coupling with mercurated chloroanilines and α- and β- naphthylamines (compounds 9-12), however, exhibit enhanced activities. The M. E. D. in case of acetoxymercuri-*p*-chloroaniline (vide Part VI, this *Journal*, 1952, **29**, 779) is 1: 100,000, but on coupling it with benzene diazonium acetate solution, the M. E. D. rises to 1 : 450,000. Such pronounced improvement is not, however, observed with Staphylococcus where the maximum activities lie in the region of 1: 40,000 to 1: 80,000 (compounds 9-12). The activities of the azo dyes prepared with other mercurated amines, excepting those with mercurated nitroanilines (compounds 2-5), are between 1: 20,000 and 1: 30,000, while those of the dyes prepared with mercurated phenols (compounds 14, 16-18, 20-22) are between 1: 10,000 and 1:20,000, even of those whose Hg contents are high.

TABLE I

R = acetoxymercuri ; A = azobenzene ; N = naphthylamine.

Name of the azo dye.	% Hg		M.p.	% Yield.	Colour.	M. E. D.	
	Found.	Calc.				E. Coli.	Staphylococcus.
1. 2′-Amino-5′-R-A (*o*-R-aniline)	44.0	43.9	Above 260°	75	Orange	1 :15,000	1 :15,000
2. 4′-Amino-3′-R-A (*p*-R-aniline)	43.5	43.9	,, ,,	70	Greyish yellow	1 :15,000	1 :15,000
3. 2′-R 4′-amino-5′-methyl-A (R-*o*-toluidine)	42.5	42.7	,, ,,	74	Yellow	1 :30,000	1 :20,000
4. 2′-Amino-3′-R-5′-methyl-A (R-*p*-toluidine)	42.6	42.7	101°	83	Grey	1 :30,000	1 :20,000
5. 2′-Methyl-4′-amino-5′-R-A (R-*m*-toluidine)	43.0	42.7	280°	70	Brownish red	1 :25,000	1 :15,000
6. 2′-Amino-3′-nitro-5′-R-A (*p*-R-*o*-nitroaniline)	39.6	40.0	260°	60	Yellowish orange	1 :10,000	1 : 8,000

TABLE I (*contd.*)

	Compounds.	%Hg					M.E.D.	
		Found.	Calc.	M.p.	%Yield	Colour.	E. Coli.	Staph.
7.	2′-Amino-3′ :5′-di-R-4′-nitro-A (*o*-*p*-di-R-nitroaniline)	52.5	52.8	252°	66	Orange	1 :25,000	1 :15,000
8.	2′-Amino 3′-R-5′-nitro-A (*o*-R-*p*-nitroaniline)	40.6	40	Above 260°	60	Do	1 :10,000	1 : 8,000
9	2′-Amino-3′-R-5-chloro-A (*o*-R-*p*-chloroaniline)	40.9	41	210°	62	Yellow	1 :450,000	1 :40,000
10.	2′-Amino-3′-chloro-5′-R-A (*p*-R-*o*-chloroaniline)	40.5	41	,, 280°	66	Brownish red	1 :400,000	1 :40,000
11.	4′-(Phenylazo)-R-β-N (R-β-N)	40 0	39.6	95°	50	Chocolate	1 :300,000	1 :80,000
12.	2′-(Phenylazo)-4′-R-α-N (2′ :4′-di-R-α-N)	39.0	39.6	,, 280°	75	Dark chocolate	1 :450,000	1 :80,000
13.	2′-Amino-anhydro-4′-carboxy-5′-hydroxy-mercuri-A (mercurated *m*-aminobenzoic acid)	46	46.6	,, 250°	85	Orange	1 :20,000	1 :12,000
14.	2′-Hydroxy-5′-R-A (*p*-R-phenol)	44	43.9	,, 250°	80	Orange	1 :10,000	1 : 6,000
15.	2′-Hydroxy-3′ :5′-di-R-A (2 :4-di-R-phenol)	60	59.9	254°d	62	Dark red	1 :20,000	1 :15,000
16.	5′-R-2′-hydroxy-3′-methyl-A (R-*o*-cresol)	43	42.6	235°	80	Brownish red	1 :25,000	1 :15,000
17.	2′-Hydroxy-(3′-R-5′-methyl-A R-*p*-cresol)	42.2	42.6	250°	80	Scarlet-red	1 :10,000	1 : 8,000
18.	2′-Hydroxy-3′-chloromercuri-5′-methyl-A (chloromercuri-*p*-cresol)	45	44.8	252°	80	Orange-red	1 :20,000	1 :15,000
19.	Anhydro-2′ : 3′-dihydroxy-mercuri-5′-methyl-A (2 :6-di-R-*p*-cresol)	49.4	48 8	250°	85	Red	1 :20,000	1 :15,000
20.	2′-Methyl-4-hydroxy-5′-R-A (R-*m*-cresol)	42.5	42.6	Above 270°	80	Orange-red	1 :8,000	1 : 6,000
21.	2′-Hydroxy-3′-R 5′-chloro-A (R-*p*-chlorophenol)	41	40.6	220°	75	Brownish red	1 :15,000	1 :12,000
22.	4′-Phenylazo-1′-R-β-naphthol (R-β-naphthol)	40	39.5	128°	80	Crimson-red	1 :15,000	1 :10,000
23.	4′-(Phenylazo)-1′-hydroxy-2-R-α-N (2 :4-di-R-α-naphthol)	39.5	40	Above 280°	66	Red	1 :20,000	1 :15,000
24.	4′-Hydroxy-anhydro-3′-carboxy-5′-hydroxymercuri-A (mercurated salicylic acid)	45	45.4	,, 260°	80	Red	1 :15,000	1 :10,000
25.	(2′ :-4′-Dihydroxy-anhydro-3′-carboxy-5′-hydroxymercuri-A (mercurated resorcylic acid)	43.5	43.9	Above 250°	75	Dark red	1 :12,000	1 : 8,000
26.	3′-R-4′-hydroxy-5′-amino-α-A (mercurated salicylamide)	40.5	40.1	215°	75	Orange	1 :10,000	—
27.	2′-Hydroxy-3′-*iso*propyl-5′-R-6′-methyl-A (mercurated thymol)	39	38.7	145°	80	Red	1 :15,000	1 :10,000

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PREPARATION OF ANTIBACTERIALS FROM ORGANO-MERCURIALS. PART IX. MERCURATED AZO DYES DERIVED FROM DIAZOTISED ACETOXYMERCURI-ANILINE

By S. S. Guha Sircar and Mahendra Kumar Rout

Preparation and antibacterial action of mercurated azo dyes, prepared from diazotised acetoxymercuri-aniline, have been described. The maximum activity noted in this series is 1 :500,000. Certain general conclusions have been drawn in regard to bactericidal activity and constitution.

In the present investigation attempt has been made to study the activity of a group of mercurated azo dyes derived from acetoxymercuri-aniline with the following structural changes which involve the presence of (*i*) acetoxymercuri group in the diazotised half without there being any Hg on the coupled half, (*ii*) acetoxymercuri and chloromercuri groups in both halves, (*iii*) naphthalene nucleus in the coupled half with Hg only in the diazotised half, (*iv*) one or two of groups such as OH, NO_2, COOH, SO_3H, NH_2 and acetoxymercuri, in different positions of the benzenoid and naphthalene nucleus of the coupled half with Hg in the diazotised half.

The compounds were prepared by the methods of Dimroth and Jacobs and Heidelberger (vide Part VIII, this *issue*, p. 361).

p-Acetoxymercuri-aniline, dissolved in dilute acetic acid, was diazotised and coupled with an alkaline solution of a mercurated phenol. The dye separated out on the addition of acetic acid. The reaction mixture was allowed to stand for 1 hour and then the precipitate was filtered. If the coupled part was an amine, coupling had to be done in acetic acid medium. Sodium acetate was added to facilitate precipitation. The dyes were purified by crystallisation from dilute acetic acid.

The properties and analyses of the resulting dyes are summarised in Table I which also gives the results of biological tests conducted in the same manner as described in previous parts.

To bring out clearly the influence of different substituents on the activities, the names of the coupling reagents have been put in parenthesis.

Table I

A stands for azobenzene and N, for naphthylamine ; R=acetoxymercuri.

Name of the azo dye.	% Hg.		M.p.	Yield.	Colour.	M.E.D.	
	Found.	Calc.				E. Coli.	Staphylo.
1. 4-R-4′-amino-A (aniline)	44	43.9	208°	68%	Greyish orange	1 :16.000	1 :12,000
2. 4-R-3′-methyl-4′-animo-A (*o*-toluidine)	42.5	42.7	196°		Orange dye	1 :20,000	1 :12,000
3. 4-R-3′-chloro-4′-amino-A (*o*-chloroaniline)	41.2	40.9	Above 270°	60	Greyish orange	1 :30,000	1 :16,000

TABLE I (*contd.*)

	Azo dyes.	%Hg					M.E D.	
		Found.	Calc.	M.p.	% Yield	Colour	E. Coli.	Stophalo.
4.	4-R-2′-amino-5′-chloro-A (*p*-chloroaniline)	40.5	40.9	242°	70	Yellowish green	1 :30,000	1 :18,000
5.	4-R-phenyl-*β*-azo-*α*-N (*α*-N)	40.0	39.7	188°	83	Crimson-red	1 :25,000	1 :15,000
6.	4-R-phenyl-*α* azo-*β*-N (*β*-N)	39.0	39.7	218°	84	Red	1 :25,000	1 :15,000
7.	4-R-3′-carboxy-4 -amino-A (anthranilic acid)	40.0	40.3	Above 270°	74	Orange-red	1 :6,000	1 :5,000
8.	4-R-2′-carboxy-4 amino-A (*m*-aminobenzoic acid)	40.5	40.3	Above 270°	64	Greyish yellow	1 :6,000	1 :4,000
9.	4-R-2′-amino-5′-carboxy-A (*p*-aminobenzoic acid)	40.7	40.3	Above 270°	76	Orange	1 :5,000	1 :4,000
10.	4 :3′ Di-R-2′-amino-5′-methyl-A (R-*p*-toluidine)	55.0	55.2	160°	65	Grey	1 :150,000	1 :16,000
11.	4-R-2′-amino-A-5-sulphonic acid (sulphanilic acid)	38 0	37.5	Above 250°	70	Dark red	1 :8,000	1 :6,000
12.	4 :3′-Di-R 2′-amino-5-chloro-A (*o*-R-*p*-chloroaniline)	54.0	53.0	150°	66	Ash colour	1 :500,000	1 :18,000
13.	4 :5′-Di-R 2′-amino-5-nitro-A (*p*-R-*o*-nitroaniline)	53.0	52.9	Above 250°	66	Purple	1 :40,000	1 :15,000
14.	4 :3′ :5-Tri-R-2′-amino-4′-nitro-A (*o*-*p*-di-R-*m*-nitroaniline)	58.0	59.2	Above 260°	70	Orange	1 :50,000	1 :20,000
15.	2′-(4-R-phenylazo)-4′-R-*α*-N (2 :4-di-R-*α*-N)	53.2	52.6	210°		Chocolate	1 :200,000	1 :18,000
16.	4 :3′-Di-R-2-amino-5-nitro-A (*o*-R-*p*-nitroaniline)	52.5	52.9	Above 260°			1 :40,000	1 :18,000
17.	2-(4-R-phenylazo)-*β*-naphthol (*β*-naphthol)	39.9	39.7	180°	85	Orange-red	1 :12,000	1 :15,000
18.	2-(4′-R-phenylazo)-*α*-naphthol (*α*-naphthol)	40.0	39.7	192°	86	Crimson-red	1 :12,000	1 :25,000
19.	4-R-2′ :4′-dihydroxy-A (resorcinol)	43.0	42.6	Above 250°	80	Brown	1 :4,000	1 :25,000
20.	4-R-3′-nitro-4′-hydroxy-A (*o*-nitrophenol)	40.5	40 0	Above 250°	52	Orange	1 :6,000	1 :25,000
21.	4-R-3-carboxy-4′-hydroxy-A (salicylic acid)	39.8	40.1	Above 250°	64	Yellow	1 :16,000	1 :12,000
22.	4-R-2′-hydroxy-5′-carboxy-A (*p*-hydroxybenzoic acid)	39.6	40.1	Above 250°	62	Red	1 :8,000	1 :6,000
23.	4-R-phenylazo-thiosalicylic acid (thiosalicylic acid)	39.3	38.9	165°	61	Greyish yellow	1 :15,000	1 :50,000
24.	4-R-2 :3′-dihydroxy-5 carboxy-A (protocatechuic acid)	40.0	38.9	Above 250°	56	Red	1 :12,000	1 :8,000
25.	4-R-anhydro-2 :3-dihydroxymercuri-5-methyl-A (2 :6-R-*p*-cresol)	59.4	60.0	Above 250°		Violet	1 :18,000	1 :8,000
26.	4 :3′ :5′-Tri-R 2′-hydroxy-A (di-R-phenol)	61.5	61.9	Above 250°	55	Red	1 :12,000	1 :10,000
27.	4-R-2′ hydroxy-3′-chloromercuri-5′-methyl-A (chloromercuri-*p*-cresol)			140°	65	Ash-coloured	1 :20,000	1 :15,000
28.	4′-(4-R-phenyazo)-1-R-*β*-naphthol (R-*β*-naththol)	53.0	52.5	145°	80	Crimson-red	1 :16,000	1 :10,000

A study of the antibacterial data shows that structural alterations as effected here do not produce any significant effect on the bactericidal properties. The bactericidal activities of azo dyes obtained by coupling with unmercurated chloroanilines, naphthylamines, aminobenzoic acids, sulphanilic acid, and several phenols show a gradation which may be roughly put in the following order:

Chloranilines (comps. 3, 4) > napthylamines (comps. 5, 6) > toluidines (comp. 2) > aniline (comp. 1) > naphthols, salicylic acid, thiosalicylic acid (comps. 17, 18, 21, 23) > aminobenzoic acid, sulphanilic acid (comps. 7, 8, 9, 10, 22) > nitrophenols and dihydric phenols (comps 19, 20).

Another fact also stands out prominently. In case of dyes having acetoxymercuri or chloromercuri groups on both diazotised and coupled halves, activity is very slightly increased by increased mercury content when the coupled part is a mercurated phenol (comps. 13, 14, 25, 26). The bactericidal values, however, become pronouncedly high, as much as 1:500,000, when the coupled half is a mercurated amine but not a mercurated nitroamine (comps. 13, 14, 15). The gradation of bactericidal values of azo dyes having Hg and NH_2 groups on both sides without any acidic groups like COOH, SO_3H and NO_2 is of the order :

Mercurated chloroaniline > mercurated naphthylamine (comp. 16) > mercurated toluidine (comp. 11) > merturated phenols, nitrophenols, hydroxy-acids, amino-acids (comps. 25, 26, 27, 28)

Whenever negative groups or phonolic groups exist,even if Hg is present in both diazotised and coupled parts, the activities rarely exceed 1:25,000. (comps. 7, 9, 17-23).

In respect of staphylococcus aureus, a similar relationship is found with the only difference that the M.E.D. in no case exceeds 1:25,000. Dyes prepared by coupling with thiosalicylic acid, naphthols and resorcinol exhibit higher bactericidal activities.

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PREPARATION OF ANTIBACTERIALS FROM ORGANO-MERCURIALS. PART X. MERCURATED AZO DYES DERIVED FROM DIAZOTISED ACETOXYMERCURI-*p*-CHLOROANILINE

BY S. S. GUHA SIRCAR AND MAHENDRA KUMAR ROUT

Preparation and antibacterial action of mercurated azo dyes, prepared from diazotised acetoxymercuri-*p*-chloroaniline have been described. The bactericidal values are markedly enhanced by introduction of the chlorine atom.

The present paper deals with the study of the activity of a group of mercurated azo dyes dervied from acetoxymercuri-*p*-chloroaniline.

EXPERIMENTAL

The dyes were prepared by the methods of Dimroth and Jacobs and Heidelberger (vide Part VIII, this *issue*, p. 361). Acetoxymercuri-*p*-chloroaniline was diazotised in acetic acid medium and coupled with a number of amines (also in acetic acid solution) or with phenols (in alkaline medium).

The properties and analyses of the resulting dyes are summarised in Table I which also gives results of biological tests, conducted as described before. To bring out clearly the relation between chemical structure and bactericidal activity, the names of the coupling reagents have been put in parenthesis.

TABLE I

A=Azobenzene. N=naphthylamine. R=acetoxymercuri.

Name of the azo dye.	% Hg.		M.p.	Yield.	Colour.	M. B. D.	
	Found.	Calc.				B. Coli.	Staphylo.
2-R-4-chloro-4′-amino-A (aniline)	40.5	40.9	198° (d)	50%	Orange-red	1 : 50,000	1 :15,000
2-R-4-chloro-2′-amino-5-methyl-A (*o*-toluidine)	40.0	39.6	205° ,,	80	Crimson-red	1 : 75,000	1 :25,000
2-R-chloro-2-amino-5′-methyl-A (*p*-toluidine)	39 0	39.6	200° ,,	50	Greenish yellow	1 : 75,000	1 :25,000
2-R-3 :4-dichloro-4′-amino-A (*o*-chloroaniline)	38.0	38.1	185° ,,	50	Crimson-red	1 :100,000	1 :20,000
2-R 4 :5′-dichloro-2′-amino-A (*p*-chloroaniline)	37.6	38.1	Above 250°	46		1 :100,000	1 :20,000
2′-(4-Chloro-2-R-phenylazo)-α-N (α-N)	37·5	37.1	,,	50	Crimson-red	1 :200,000	1 :25,000
4-Chloro-2-R-phenylazo-β-N (β-N)	37.0	37.1	158° (d)	70	Dark red	1 :150,000	1 :20,000
2′-Amino-4-chloro-2 :5-di-R-A (*p*-R-aniline)	54.0	53.5	Above 250°	66	Orange	1 :250,000	1 :25,000
4 :5′-Dichloro-2 :3′-di-R 2′-amino-A (R-*p*-chloroaniline)	52.4	51.9	,, ,,	65	Red	1 :500,000	1 :25,000
4-Chloro-2-R-phenylazo-R-β-N (R-β-N)	50.0	50.1	,, ,,	63	Crimson-red	1 :200,000	1 :20,000
4′-R-(4-chloro-2-R-phenylazo)-α-N (R-α-N)	49.5	50.1	,, ,,	66	Dark red	1 :300,000	1 :30,000
2-R-2′-amino-4-chloro-5′-chloromercuri-A (chloromercuri-aniline)	55.0	55.2	,, ,,	72	Red	1 :300,000	1 :30,000
2-R-4-chloro-2′-methylamino-5′-chloromercuri-A (chloromercuri-methylaniline)	54.5	54.3	,, ,,	72	Dark red	1 :400,000	1 :30,000
2-R-4-chloro-2′-dimethylamino-5-chloromercuri-A (chloromercuri-dimethylaniline)	53.0	53.1	,, ,,	78	Dark red	1 :400,000	1 :30,000
4′-Chloromercuri-(2-R-4-chlorophenyl-β-azo) α-N (chloromercuri-α-N)	52.0	51.6	,, ,,	66	Crimson-red	1 :450,000	1 :30,000
(4-Chloro-2-R-phenyl-β-azo)-α-naphthol (α-napthol)	36.3	37.0	158° ,,	70	Chocolate	1 : 18,000	1 :30,000
(Chloro-2-R-phenyl-α-azo)-β-naphthol (β-naphthol)	37 1	37.0	172° (d)	79	Crimson-red	1 : 20,000	1 :20,000
(4-Chloro-2-R-phenylazo) thiosalicylic acid (thiosalicylic acid)	36.0	36.3	150° (d)	80	Orange.	1 : 30,000	1 :30,000
(4-Chloro-2-R-phenylazo)-salicylic acid (salicylic acid)	37.0	37.4	Above 280° ,,	80	Chocolate	1 : 20,000	1 : 8,000
(4-Chloro-2-R-phenylazo)-*o*-nitrophenol (*o*-nitrophenol)	38.0	37.4	,, ,, ,,	80	,,	1 : 10 000	1 :20,000
(4-Chloro-2-R-phenylazo)-resorcinol (resorcinol)	39·9	39·4	,, ,, ,,	78	,,	1 : 6.000	1 : 8,000

It appears that the effect of a Cl atom in the diazotised nucleus is quite significant as can be seen by comparing the activities of the members of this group with those given in Part IX (this *issue*, p. 364). The bactericidal values are considerably enhanced by introduction of a Cl atom. The present series of azo dyes show the following graded relationship, only the coupled part is mentioned, as the diazotised part is the same in all cases.

Chloroanilines > naphthylamines > toluidines > aniline, naphthol, salicylic, aminobenzoic acids and sulphanilic acid > nitrophenol and resorcinol.

The gradation of bactericidal values of azo dyes having Hg and NH_2 groups on both sides without acidic groups (like COOH, SO_3H and NO_2 groups) is of the order mercurated chloroanilines > mercurated naphthylamine > mercurated toluidines > mercurated phenols, hydroxy-acids, amino-acids and sulphanilic acid.

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COMPLEX COMPOUNDS OF COPPER AND NICKEL WITH PHENYLMETHYLBIGUANIDE

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Contrary to the expectation, because of the so-called 'size factor', only one form of phenylmethylbiguanide complexes has, however, been actually obtained. Besides copper and nickel phenylmethylbiguanidinium hydroxides, a number of other salts, namely, sulphate, chloride, bromide, iodide and nitrite have been described. The complexes appeared to be less stable than the analogous complexes with biguanide.

Rây and Chakravarty (this *Journal*, 1941, **18**, 609) have obtained more or less definite evidences regarding *cis-trans* isomerism in the case of nickel phenylbiguanidines. In the case of the copper complexes the results were not so convincing. Copper complexes with naphthylbiguanide, investigated by Rây and Ray (*ibid.*, 1944, **21**, 163), only exhibited different modifications in the case of the complex chloride. Nickel naphthylbiguanidinium base could, however, be obtained in three different modifications. Phenylmethylbiguanide complexes of copper and nickel are expected, because of the so-called 'size' or 'weighting factor', to exhibit this property in a greater degree. Hence, a study of the preparation and properties of these compounds was undertaken. Contrary to expectation, only one modification of complexes could be isolated. Attempt to transform them into other modifications with the effect of temperature, humidity and acidity was not successful.

Experimental

Phenylmethylbiguanide, $C_9N_5H_{13}$, was prepared according to the method of Cohn (*J. prakt. Chem.*, 1911, **84**, 394) by boiling for 2 hours a mixture of dicyandiamide (18 g.), methylaniline (10 g.), 25% HCl (13 g.) and water (10 g.). The reaction mixture, after cooling, was treated with 33% NaOH solution when a dark oily liquid separated out. On extraction with ether white needles of the organic base separated out. The crystals were found to be very hygroscopic. An aqueous solution of the base gave coloured precipitates with salts of copper, nickel, cobalt and palladium.

Copper-phenylmethylbiguanidinium Salts

Copper-phenylmethylbiguanidinium Sulphate.—The oily liquid, as obtained in the reaction described above, was separated. To this was added ammoniacal copper sulphate solution when rose-red precipitate of the complex copper base was obtained. This crude product was filtered, washed and then dissolved in dilute hydrochloric acid and the base reprecipitated with freshly prepared sodium hydroxide solution. This was filtered and washed and then treated with solid ammonium sulphate on the water-bath till there was no more evolution of ammonia. The resulting pink solution was filtered and cooled, when pink crystals of the complex sulphate separated out. These were

filtered, washed with cold water and alcohol and dried in air. The compound, when dry, is difficultly soluble in water, but slightly soluble in alcohol. {Found: Cu, 10.41; SO_4, 15.76. $[Cu(C_9N_5H_{13})_2]SO_4.4H_2O$ requires Cu, 10.36; SO_4, 15.64 per cent}.

The *chloride* was prepared by the action of ammonium chloride on the complex base on the water-bath. A violet solution was left after the evolution of ammonia, from which violet crystals separated on cooling. The product was recrystallised from hot water, filtered, washed with alcohol and dried in air. It is appreciably soluble in warm alcohol. {Found: Cu, 11.88; Cl, 13.25. $[Cu(C_9N_5H_{13})_2]Cl_2.H_2O$ requires Cu, 11.89; Cl, 13.28 per cent}.

The *bromide* was prepared by adding a strong solution of potassium bromide to that of the complex chloride. It forms violet crystals, moderately soluble in water, but more so in alcohol. {Found: Cu, 10.21; Br, 25.64. $[Cu(C_9N_5H_{13})_2]Br_2.H_2O$ requires Cu, 10.19; Br, 25.64 per cent}.

The *iodide* was prepared in the same way as the bromide by treating the chloride with a concentrated solution of potassium iodide. Blue-violet crystals were at once obtained which were recrystallised from hot water and dried in air. {Found: Cu, 8.34; I, 32.52. $[Cu(C_9N_5H_{13})_2]I_2.4H_2O$ requires Cu, 8.24; I, 32.51 per cent}.

The *nitrite* was obtained as reddish violet crystals by the addition of a cold concentrated solution of sodium nitrite to that of the chloride. The substance was recrystallised from hot water and dried in air. It is soluble in alcohol. {Found: Cu, 11.82; N, 30.92. $[Cu(C_9N_5H_{13})_2](NO_2)_2$ requires Cu, 11.78; N, 31.06 per cent}.

The *complex base* was prepared in a pure state by the addition of a freshly prepared solution of sodium hydroxide to that of the pure chloride. The dull brick-red precipitate was washed by decantation with ice-cold water and then filtered, washed again with water and alcohol. It was dried in air free from CO_2. {Found: Cu, 13.21; N, 30.09. $[Cu(C_9N_5H_{13})_2](OH)_2$ requires Cu, 13.25; N, 29.19 per cent}.

Nickel-phenylmethylbiguanidinium Salts

Nickel-phenylmethylbiguanidinium Sulphate.—The complex nickel base was first precipitated by the addition of nickel sulphate solution to the oily liquid which separated when the mixture of dicyandiamide, methylaniline and hydrochloric acid, after the reaction, was treated with alkali, as described in the preparation of complex copper sulphate. The crude product was dissolved in dilute hydrochloric acid and the base was reprecipitated by the addition of sodium hydroxide. This was filtered, washed with cold water and then the moist precipitate was dissolved in dilute sulphuric acid. A light cream-coloured solution was obtained which on keeping in the cold deposited light orange-yellow precipitate. These were filtered, washed with cold water and alcohol and dried in air. {Found: Ni, 10.33; SO_4, 16.98. $[Ni(C_9N_5H_{13})_2]SO_4.2H_2O$ requires Ni, 10.24; SO_4, 16.76 per cent}.

The *chloride* was prepared in the same way as the sulphate by neutralising the base with dilute hydrochloric acid. From the greenish yellow solution, so obtained, light yellow needle shaped crystals separated slowly in the cold. These were filtered, washed with cold water and alcohol and dried in air. {Found: Ni, 10.41; Cl, 12.49.

$[Ni(C_9N_5H_{13})_2]Cl_2.3H_2O$ requires Ni, 10.37; Cl, 12.55 per cent}. The substance easily undergoes hydrolysis in aqueous solution giving a slimy, light orange precipitate.

The *bromide* was obtained as dirty brown plates by the addition of a concentrated solution of potassium bromide to that of the complex chloride in the cold. The crystals were washed with cold water and alcohol, and dried in air. {Found: Ni, 9.18; Br, 25.61. $[Ni(C_9N_5H_{13})_2]Br_2.2H_2O$ requires Ni, 9.22; Br, 25.11 per cent}.

The *iodide* was prepared by triturating the complex base with ammonium iodide, when ammonia was evolved and a yellowish solution resulted, which in the cold slowly deposited yeliow crystals of the iodide. These were filtered, washed with water and alcohol, and dried in air. A gummy product was obtained when an attempt was made to precipitate the iodide by the action of KI to the complex chloride solution. {Found: Ni, 8.19; I, 34.40. $[Ni(C_9N_5H_{13})_2]I_2.2H_2O$ requires Ni, 8.03; I, 34.74 per cent}.

The *complex hydroxide* was prepared in the same way as the copper base. The substance, which is light yellow in colour, was washed with cold water and alcohol and dried in air free from CO_2. {Found: Ni, 12.28; N, 30.12. $[Ni(C_9N_5H_{13})_2](OH)_2$ requires Ni, 12.36; N, 29.70 per cent}.

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